

STERNAL PUNCTURE

A METHOD OF CLINICAL AND
CYTOLOGICAL INVESTIGATION

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WITH A FOREWORD BY

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FOREWORD

STERNAL PUNCTURE is the generally accepted means by which myeloid tissue may be studied during life. It is one of the most modern of the techniques included under the term "clinical pathology." A little hesitant at first, it has now developed into a full-blown method of investigation.

By the help of sternal puncture three things are being accom-

states of the body are being observed. All these aspects of the subjects are covered in Dr. Piney's present monograph.

The limits of usefulness of the new technique cannot yet be stated. They must await much more exploitation of the method than has so far been undertaken.

the liver occur as possible examples.

The authors have avoided dogmatism and have wisely kept such knowledge as we have in this field in a fluid state, where it should be allowed to remain whilst the technique is in its early history and the body of ascertained facts relatively small. Equal care has been shown in handling the nomenclature.

The book is timely because it summarises the present position of the subject and acts as a manual for investigators, whether their approach is on the pathological or on the clinical side.

The popularity which has been achieved by Dr. Piney's previous books in hæmatology may safely be predicted for this new work also.

HORDER

PREFACE TO FOURTH EDITION

THE need for a fourth edition of this book suggests that the mode of presentation meets a real need, and we have, therefore, not altered the style by attempting to produce a complete review of the subject. There are many excellent text-books of hæmatology, some of which proceed from the consideration of the peripheral blood to a discussion of the marrow, while there are others in which the opposite course is adopted, but our aim has been (and still is) to present the essential features of the bone-marrow in health and disease. For this reason, we have not increased the size of the book, but have extended our consideration of the relevant literature, calling attention to such papers as contain an adequate bibliography.

For those who require extensive references to the literature, we would suggest study of the monographs cited at the bottom of this page.

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INTRODUCTION

BLOOD examination, which has become an important adjuvant in both diagnosis and prognosis has always suffered from the weakness of depending for its value on the inferences drawn from it. Knowledge of the concomitant changes in the formative tissues has been scanty. Attempts have often been made to correlate blood changes with those in the marrow, but the difficulties have been great. First, the blood has been examined by the film method, whereas the marrow has been examined in sections when the cells look very different. Secondly, marrow sections can usually only be obtained from post mortem material and will then reveal only the terminal state.

Marrow puncture is a convenient method of examining the formative tissue during life, and it can, without difficulty, be performed several times on the same patient. There can be no doubt that it has led to a much deeper knowledge of the factors on which the characters of the blood picture depend.

The blood is, of course, not a tissue in the usual sense of that word. It is a product of a number of organs of which the bone marrow is the most important, and yet the blood is not a secretion of the hæmopoietic tissue in the same sense that urine is a secretion of the kidneys. It does not consist of constituents that have been abstracted from the body as a whole but of portions of the parent tissue itself. It is for this reason that it is convenient to conceive of the whole mass of circulating cells together with those in the formative tissues as forming an organ. Thus Boycott spoke of the *erythron* as the organ composed of the whole collection of red cells and their precursors in the body. A similar concept, the *leucon*, is applicable to the white cells, and the *thrombon* to the platelets.

These organs are not totally independent of one another because the primitive parent cells of both the red and the white elements are probably identical, in other words, at the earliest stage, the *erythron*, the *leucon* and the *thrombon* meet. For this reason we must make use of the conception of the *hæmaton*, which is then the whole of the blood and the blood forming system. Thus, examination of the marrow is so important because it reveals something more than the characters of the parent tissue of the blood, it shows us an integral part of the *hæmaton*.

It was the improper abstractive separation of the circulating blood from the formative tissues that led in the past to a deep and fundamental cleavage in the hæmatological world. Thus, there were two main schools of thought—the monophyletic and the polyphyletic. The former contended that all the different types of blood cells were derived from a common ancestral form, which was variously known as the lymphoidocyte, the hæmocytoblast, and about eighty other synonyms. The polyphyletists, on the other hand, asserted that there were several stem cells, each one with irreversibly determined potentialities. The number of such elements was a matter of discussion among the several groups of this school, but all were agreed that the monophyletists were wrong.

This controversy, which, in the past, filled thousands of pages, is no longer acute because our knowledge of the marrow has increased. In the past, most observations on the blood-forming organs were carried out by professional histologists, who naturally made use of their customary technique, that is to say, fixation, embedding in paraffin or celloidin, section cutting and staining, and most, if not all the histologists were adherents of the monophyletic view. The clinical hæmatological workers, on the other hand, based their conclusions on observations made on stained films, and very many of them were polyphyletists.

The divergence of views was to a very great extent dependent upon these differences of technique, because cells which appear large and full of cytoplasmic and nuclear detail, when seen in films, are small and difficult to recognise in fixed sections. Marrow puncture has enabled us to use the same technique for marrow tissue as for blood, and, as a result, the old controversy has almost died down.

Various methods of obtaining marrow during life were used in the past. Thus the femur was trephined and marrow extracted, or the tibia was similarly treated, and from both these methods a good deal of information was obtained. There are two main objections to such procedures. They are in the nature of major surgical operations and cannot be repeated frequently on the same patient. Secondly, in adult life the red marrow in the limbs is confined to small areas at the upper ends of the humeri and femora, the more distal parts of these bones and the more distal bones of the limbs containing only fat. In diseases, in which there is excessive production of cells, formative marrow may spread into all the bones. Thus,

in advanced leukaemia and pernicious anaemia, valuable information could be obtained from the tibial bone-marrow, but in health and more acute maladies only fatty tissue was present. Obviously then, it would be a great advantage to obtain the specimen from a bone that is always filled with active marrow, such as the sternum. Since 1929, when Arinkin introduced the method of sternal puncture, this has been the usual technique.

It may be mentioned that marrow puncture is not only of value in the investigation of most of the disorders of the blood and in many diseases due to protozoa—it has been found that it may assist in the bacteriological diagnosis of typhoid fever. Rogge obtained a positive culture from the marrow in 60 per cent of cases, and, in 9 cases, was successful in this way when all other attempts at culture had been unsuccessful. In the first week of the disease, 4 cultures out of 4 were positive, while only 19 out of 30 blood cultures were so. In the second week, 15 out of 21 sternal cultures were positive, against 9 out of 38 blood cultures. Thus, it would seem that typhoid fever cannot be excluded if the sternal marrow has not been cultured, even although all other tests have proved negative.

The material obtained by marrow puncture has even been used therapeutically. Morrison and Samwick claimed good results from intra-sternal injection of marrow juice in two cases of aplastic anaemia, but others have failed to obtain similar results. Schretzenmayer injected sternal marrow intramuscularly as a treatment for various anaemias, and believed that the method was of value—the evidence presented is, however, poor.

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STERNAL PUNCTURE

CHAPTER I

THE MYELOGRAM

THE recognition of the various types of cells in blood films needs a good deal of practice, and it is quite impossible to interpret the appearances in marrow films without a sound knowledge of the cytology of the peripheral blood. This is, of course not surprising, because the marrow cells are either blood cells or are their precursors, and, for this reason we can use a similar terminology to that used in ordinary hæmatology.

Schilling coined the name *hæmogram*, to designate the qualitative blood picture, and this is a much sounder concept than is the ordinary term "differential count". The term, *myelogram*, can with equal reason be applied to the total marrow picture. Just as experience has given us knowledge of the normal percentages of the different types of cells in the blood in health and in a great variety of diseases, so observation has led to a knowledge of the marrow picture, the myelogram in health and disease.

The hæmogram is naturally not an absolutely accurate picture of the cellular composition of the peripheral blood, because it is based on random sampling, both of the whole blood and even of the specimen taken for examination. The myelogram is subject to the same sources of error, but is also less accurate than the hæmogram for another reason. A varying proportion of the cells in the marrow cannot be classified either because of pathological changes in their structure, or, even in health by being in a more or less differentiated state, prior to during or immediately after mitosis. Even so, the myelogram is of great value, but has to be recorded in a more detailed manner than the hæmogram, because there are more classes of cells in the marrow than in the blood. Then again, another difficulty is that marrow films cannot be evenly spread, there is admixture of a certain amount of fat and the cells are not floating free, as in blood but are more or less aggregated into clumps.

Both for purely scientific and for clinical purposes, the myelogram and the hæmogram should be investigated at approximately the same time, and completeness would, of course, require examination of puncture fluid from the other hæmopoietic organs, spleen and liver.

more than describe its structure. The cytoplasm, which is devoid of granules, is distinctly basophilic, and varies considerably in amount. The nucleus is a good deal denser than that of the hæmohistioblast, but has the same type of reticular structure, being composed of interlacing fibrils of basichromatin, in which one or more nucleoli can easily be seen (Plate 3).

The followers of Ferrata regard this element as being the most primitive cell that has become irreversibly fixed in the direction of hæmopoiesis—unlike its alleged ancestor, the hæmohistioblast, it can no longer produce connective tissue. If this be so, the hæmocytoblast is the real stem-cell of the whole hæmopoietic process. Certainly, in marrow that is hyperplastic from any cause, these cells are increased in number. Thus, in the megaloblastic marrow of pernicious anæmia, in ordinary normoblastic marrow hyperplasia, in leucoblastic, and even in leukæmic marrow they are increased in number. This fact seems to justify the inference that they are fixed in their general hæmopoietic potency, but are still multi potent, inasmuch as they can give rise to either red or white cells.

On the other hand, Naegeli contended that the hæmocytoblast is not a multipotent stem cell, but that it is identical with the myeloblast—the parent of the granular leucocytes only. But his view fails to supply any explanation of the increase of these cells in all types of hyperplastic marrow, if he were right, it would be surprising to find these leucopoietic elements increased in erythroblastic reactions.

As long as we are clear in our minds about the structure of these elements, so that we can recognise them, their exact status can be left to further research, but the name, hæmocytoblast, is now so widely used that we may well adopt it.

Myeloblast. The supporters of the view that the hæmocytoblast is multipotent, distinguish it from the myeloblast (Plate 2), which they consider to be an element irreversibly determined in the direction of granulocyte formation, that is to say, they regard it as a more differentiated cell. It is, unfortunately, difficult to decide on what morphological criteria myeloblasts are to be distinguished from hæmocytoblasts. It is said that the nuclei of the former are rather less tenuous, and that the cytoplasm is less basophilic. But it is, of course, not to be expected that exact distinctions can be made, because we are dealing with a continuous process of development, not with isolated cell types.

Premyelocyte. The premyelocyte (Plate 2) is intermediate between the myeloblast and the myelocyte, that is to say, there is

no doubt that it is definitely fixed in its potencies, and its structural characters lie, as it were, midway between the two types of cell. There seem to be two grades of premyelocytes. First there are those in which the nucleus is only a little denser than that of a myeloblast and still contains nucleoli, but the cytoplasm is less basophilic and contains azurophilic granules. Most of these cells have minute granules all of the same size (*pre-neutrophilic premyelocytes*). Some have large rather scanty granules, also all of the same size (*pre-eosinophilic premyelocyte*), and finally, there are a few elements with large granules of irregular size (*pre-basophilic premyelocytes*). From these arise neutrophilic, eosinophilic and basophilic premyelocytes in which the nucleus has become a good deal denser and the nucleoli are obscured, while the granules have reached their definitive staining reactions.

Myelocytes These cells are the stock from which most of the mature granulocytes arise in health. In the marrow they vary greatly in size, some being little larger than lymphocytes, while a few are as much as 26 microns in diameter. This variation in size is even greater than that seen in the blood in chronic myeloid leukaemia, although in the past it was supposed that the variations in that disease were due to the morbid process. The nucleus of the myelocyte is circular but the edge may be slightly indented as if by pressure of the granules that fill the cytoplasm. The chromatin still has a reticular arrangement resembling that of the myeloblast, but condensations and small nodules can be seen in the stem. The cytoplasm is slightly basophilic but is difficult to see because it is almost filled with granules, which, at this stage, have, of course, reached their definitive staining reactions (Plate 2). The eosinophil myelocytes present difficulty, because, although many of them contain mature eosinophil granules, others present a mixture of granules, some being basophilic and others eosinophilic.

A variable number of such elements is always found in the marrow, but the exact steps in their origin from premyelocytes are unknown. It is possible that some of the cells in the marrow, which contain only basophilic granules, are really the very earliest stage of eosinophils. If this is so, it would account for the fact that the marrow always contains more basophils than one would expect from the appearances of the peripheral blood.

Metamyelocytes These are intermediate between myelocytes and mature polymorphs and can be divided into juvenile forms, with very slight indentations of the nucleus, and staff forms in which

the nucleus is deeply indented, rather like a band (Plate 2). It is at the stage of the staff form in the marrow that abnormalities of structure occur in pernicious anæmia, some of the cells being as much as 30 microns in diameter.

Polymorphonuclears As seen in films of normal marrow, these cells are identical with those found in the blood (Plate 2), but, even when the blood polymorphs appear normal, atypical forms may be seen in the marrow. Thus, in chronic myeloid leukæmia, very large ones may be seen, and in pernicious anæmia equally gigantic forms, mainly neutrophilic, with most complicated nuclei, are common in untreated cases.

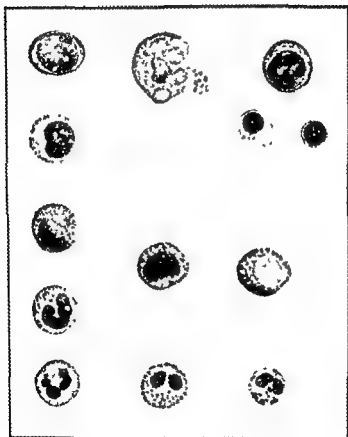
Lymphocytes These are not part of the myeloid tissue proper, but there seems to be no doubt that the marrow is the seat of production of a small proportion of the lymphocytes in the peripheral blood. Certainly, even in adults, it contains more large lymphocytes, which are commonly supposed to be younger forms. In rickets small aggregations of lymphocytes, sometimes even with a germ centre, may be found. It is unusual to find immature cells of the lymphocyte series in normal marrow, but in glandular fever and in lymphatic leukæmia they may be numerous.

The typical immature cell of this series is the *lymphoblast* (Plate 2), which is, so to speak, equal in its developmental potencies to the myeloblast, i.e., it is irreversibly determined in the direction of lymphocyte formation. It is about the same size as the myeloblast but with a relatively larger nucleus, which is more coarsely reticulated than that of the myeloblast. It contains nucleoli around which the chromatin is somewhat condensed as it is also at the edge of the nucleus where it forms an almost distinct membrane.

Monocytes Monocytes are scanty in normal marrow but appear to arise from the *hæmohistioblast* through the stages of monoblast and promonocyte. The monoblast resembles the myeloblast and lymphoblast in general structure, and may not be easily distinguishable from them. The promonocyte shows some indenting or folding of the nucleus with the characteristic ground glass cytoplasm of the adult monocyte, fine azurophilic granules are often visible in the cytoplasm. The adult monocytes are in no way different from those seen in the blood.

Plasma Cells. Plasma cells are scanty but invariably constituents of the marrow even in health. They increase slightly in hypoplastic conditions and a great deal in myeloma. There is still much controversy as to the origin of these cells, and it remains un-

PLATE 2



DEVELOPMENT OF WHITE CELLS

certain whether those of the blood and marrow are identical with those found in the tissues. In the marrow most of these cells are quite large (15 to 20 microns), have an excentric nucleus, in which the basichromatin has a "cart-wheel" arrangement, intense basophilic cytoplasm, and usually a pale area round the nucleus. Similar cells, in which the nucleus is in the middle (Turk cells), are also found, and, like the plasma cells, their origin is uncertain.

There is a good deal of evidence that any type of non granular blood or marrow cell may, for reasons as yet unknown, assume plasmacytoid characters so that it is probable that lymphocytic, lymphoblastic, myeloblastic and even erythroblastic plasma and Turk cells exist. It has been asserted that plasma cells have a distinct line of development from plasmoblasts, and Moeschlin's work on these cells in rubella seems to support a similar view, although he gives no criteria by which one can distinguish plasmoblasts from lymphoblasts.

Megakaryocytes. Megakaryocytes are important components of the marrow and, in spite of their great size and irregular shape, they, or parts of them, are usually recognisable in films. As they are rarely numerous, it is best to seek for them with low magnifications, and they are most likely to be found intact only near the edges and ends of films. They are from 30 to 90 microns in diameter with faintly basophilic cytoplasm in which small groups of azurophilic granules are present, mainly near the edges (Plate 2).

The nuclei present most complicated lobulation, but there does not seem to be any distinctive distribution of the basichromatin, which is mainly disposed in the form of large masses. It is possible that many of the structures, which in marrow films seem to be only parts of megakaryocytes, are really complete but immature elements which lie on the line of development from the histiocyte to the fully formed megakaryocyte. The relative simplicity of the nuclei of these *megakaryoblasts* seems to support this view.

There is much controversy about the nomenclature of the various megakaryocyte like cells that may be found in marrow films, but, of the many suggestions that have been put forward, those of Dameshek and Miller seem to have most to commend them.

These authors distinguish the following forms —

(1) *Megakaryoblasts* which are about twice the size of myeloblasts, have non-granular cytoplasm, and a rather irregular single nucleus which contains several nucleoli.

(2) *Promegakaryocytes* are about the same size as megakaryoblasts,

although much larger forms also occur. The nucleus is usually not fully lobulated. The cytoplasm is rather scanty and is distinctly basophilic. In it there are usually a few aggregations of azurophilic granules, resembling platelets, near the periphery of the cell body. There may also be a few of these cells with non-granular cytoplasmic projections and such promegakaryocytes seem to be specially common in thrombocytopenic purpura.

(3) *Lymphoid megakaryocytes* (rather a poor name) are large cells with relatively small lobulated nuclei and non-granular cytoplasm.

(4) *Megakaryocytes*

(5) *Prepolykaryocytes* are mononuclear cells, which are often seen in small clusters. The basophilic cytoplasm is vacuolated, and there is a reticulated nucleus which contains nucleoli.

(6) *Polykaryocytes* are assumed to be fused syncytia of prepolykaryocytes, and are supposed to be osteoclasts under another name. It is suggested that these cells may, by nuclear fusion, give rise to megakaryocytes—an improbable occurrence.

Red Cells. It is now almost universally assumed that all red cells, both normoblasts and megaloblasts, arise from a common ancestor—the hæmocyctoblast. We need not enter into the time-honoured discussion of the genealogical history of the red cells in general, but, as the morphological differences between normoblasts and megaloblasts are considerable, they are best described separately.

See also description on Plate 2

of the ordinary red corpuscle. In marrow films it is relatively easy to trace out every stage in this process.

The least mature element that is recognisable as definitely belonging to the red cell series is the *pro erythroblast*, which is a large cell slightly resembling the hæmocyctoblast but possessing a distinctive nuclear arrangement.

The basichromatin is no longer purely reticular, as in the hæmocyctoblast, and shows a tendency to be aggregated into almost triangular masses with a roughly radial arrangement. Pale areas, which are perhaps nucleoli, can be detected, but the cells in which these can be seen are scanty. The cytoplasm has become less intensely basophilic, and when no trace of nucleoli can be seen we have reached the next clear-cut stage—the *basophilic erythroblast*. In this the radial arrangement of the chromatin is quite distinct, and

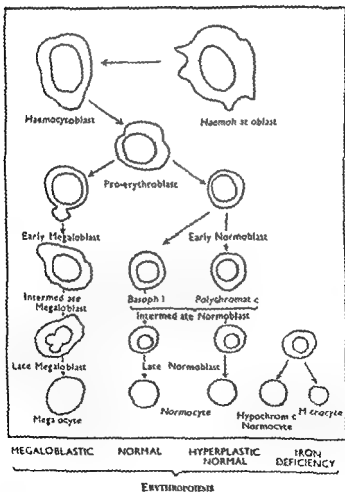
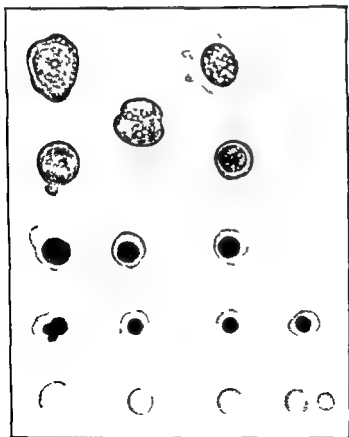


PLATE 3



ERYTHROPOIESIS

of the different names used by different authors for the same cells. In order to lessen this confusion we have constructed the accompanying table where an attempt has been made to correlate the different names used to describe the various cells, together with the authors who have used these names (p. 11).

Atypical Erythropoiesis. Lizarzi and Levinson have recorded an extremely bizarre and previously undescribed type of erythropoiesis observed in the bone-marrow of a man aged seventy-seven suffering from prostatic obstruction. Very large cells (apparently giant basophil megaloblasts) were seen which underwent repeated mitotic division of the nucleus producing a multinucleated cell which eventually split to form ordinary basophil normoblasts, these developed into ordinary basophil normoblasts which in turn developed into ordinary erythrocytes. Other similar cells behaved in the same way except that nuclear division occurred amitotically. Where division of the nucleus was incomplete large nucleated red cells were formed which, after extrusion of the nucleus, left corpuscles as large as 23μ in diameter—these corpuscles did not appear in the peripheral blood but underwent a process of fragmentation in the marrow.

Since this first report, Schwarz has studied many marrow films and found cellular gigantism and pluripolar mitosis of both the myeloid and erythroid cells. This apparently may occur in many conditions as well as in normal people. Schwarz's paper does not give the frequency with which this phenomenon occurs, but it must be comparatively rare as neither of us have ever seen it. Fig. I, re drawn after Schwarz, shows the types of cell which may be encountered. The changes apparently are not of pathological significance and the resulting monsters should be regarded as "sports."

The Normal Myelogram. It is not possible to make accurate total cell counts on the bone-marrow. Segerdahl, who tried to do so, found that in health the number varied from 10,000 to 190,000 per cubic millimetre. There can be little doubt that various factors influence the total count quite apart from the structure of the marrow itself. Thus, if only small amounts of marrow are withdrawn by sternal puncture, the total number of cells per unit volume is greater than if a large amount is withdrawn, and, even more strangely, the proportion of white cells is higher. There seems no doubt that it is only the differential count from which inferences can be drawn, and it is disappointing to find that the marrow obtained by sternal

FIG. 1



C



D



E



F



G



TABLE I *A correlation of the different names used by authors for various blood and stem cells. The description of each cell will be found on the page indicated in the left-hand column*

This Book	Alternative Name	Author
Hæmohistioblast (p. 3)	— —	Ferrata Piney
Hæmocytoblast (p. 3)	— — — Lymphoidocyte Erythrogonic Promegaloblast Megaloblast	Schulten Israels Maximow Pappenheim Helly Naegeli Doan, Cunningham and Sabin
Pro erythroblast (p. 8)	— — — Erythrogonic — Promegaloblast — Megaloblast —	Schulten Renssels and Ferrata Israels Dameshek and Valentine Jaffe Nordensen Naegeli Doan, Cunningham and Sabin Peabody
Basophilic megaloblast (p. 8)	Megaloblast — Megaloblast A	Doan, Cunningham and Sabin Peabody, Israels
Polychromatic megaloblast (p. 9)	Megaloblast. — — Megaloblast B	Ehrlich Turnbull Gilmour Israels
Eosinophil megaloblast (p. 9)	Megaloblast C	Israels
Basophil normoblast (p. 8)	Pronormoblast — Macronormoblast — Early erythroblast Normoblast A	Nordensen Naegeli Pappenheim Naegeli Doan, Cunningham and Sabin Israels
Polychromatic normoblast (p. 8)	Late erythroblast Normoblast B	Doan, Cunningham and Sabin Israels
Eosinophil normoblast (p. 8)	Normoblast Necroblast C	Doan, Cunningham and Sabin Israels

puncture before death gives a very different picture from that obtained by the same procedure within half an hour after death. In other words, the inferences we may draw from the myelogram are much less certain than those which we may draw from the ordinary blood count.

From the figures obtained by examining from 500 to 1,000 consecutive cells in marrow films, useful relationships can be revealed. Thus, in health, the proportion of white cells of all sorts to nucleated red cells varies from 5-1 to 3-1, but in the *anæmias* the proportion is much lower and the relationship may even be inverted. Then again, the proportion of granular to non-granular white cells is of some importance. Normally it is about 4-1. Again the numerical relationship of normoblasts to megaloblasts is significant, particularly in the diagnosis of pernicious *anæmia*.

In the normal bone-marrow the cells multiply by ordinary karyokinetic division, and, in films of the marrow-fluid, mitotic figures are always seen. It is important to arrive at some estimate of the percentage of dividing cells, and in which particular group of cells, *i.e.*, myeloblasts, myelocytes, etc., mitosis is most active, because from this information very useful deductions can be drawn. Japa has given the following figures for normal bone marrow, in each 1,000 nucleated cells about 15 show mitotic figures, 40 per cent of these are in the prophase, 45 per cent in the metaphase, 10 per cent in the anaphase, and 5 per cent in the telophase. The proportion of dividing leucoblasts to erythroblasts is given as 45 : 55, of myeloblasts to myelocytes 3 : 97, of early normoblasts to late normoblasts 91 : 9.

In simple hyperplasia of the leucoblastic or erythroblastic tissues following acute pyogenic infection or acute hæmorrhage, the number of mitotic figures may double itself, but there will be no significant alteration in the ratios of the dividing cells in the myeloid and erythroid groups respectively. The *leukæmias*, however, show a very different picture. Here the number of dividing cells also shows a marked increase, but a larger percentage of the mitotic figures are in the more immature cells. This is well seen in the myelogenous *leukæmias*, in the chronic forms there is an increase in the number of dividing premyelocytes, and to a much smaller degree in the myeloblasts. As the malady becomes more acute the proportion of mitotic myeloblasts rises until, in the true acute form, they are the only dividing cells seen.

NORMAL MYELOGRAM

Neutrophiles	
Myelocytes	30-35 per cent
Metamyelocytes	10-15 " "
Polymorphs	24-30 " "
Eosinophiles	
Myelocytes	1-2 " "
Polymorphs	$\frac{1}{2}$ -1 " "
Basophiles	
Myelocytes	3-6 " "
Polymorphs	1-2 " "
Premyelocytes	1-2 " "
Hæmocyto blasts, including myeloblasts	1-2 " "
Hæmohistioblasts	$\frac{1}{4}$ - $\frac{1}{2}$ " "
Lymphocytes	
Small	6-9 " "
Large	8-12 " "
Plasma cells	$\frac{1}{4}$ -1 " "
Pro erythroblasts	1-2 " "
Normoblasts	15-20 " "
Megaloblasts	2-3 " "
Megakaryocytes scanty, but invariable	

It is possible that some, if not all, the cells called megakaryoblasts in this table are really large normoblasts; but many writers state that a few genuine megakaryoblasts are present, even in health.

Much has now been written on the variations in the normal myelogram. Shapiro and Bassem have studied the sternal marrow changes during the first week of life and shown that there is a marked drop in the number of normoblastic cells at the end of the first week of life which runs parallel with the decrease in circulating reticulocytes. Diwany gives the normal picture in children from one to five years of age, and Vogel and Bassem give the myelograms of forty-one children of various ages. The hæmatology of the sternal marrow in normal women is discussed by Pitts and Packham, and the same authors and Markoff give the variations which are found during normal pregnancy (p. 54).

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CHAPTER II

THE MARROW IN LEUKÆMIA

THE marrow changes in the various forms of leukæmia are usually distinctive, and for this reason it is best to start our description with this group of diseases. It is not to be supposed that marrow puncture in the leukæmias is only of academic interest. Admittedly, in many, perhaps most, cases of leukæmia, diagnosis is possible on clinical and ordinary hæmatological grounds. Blood examination usually enables one to discover the type of leukæmia and also gives some indication of the severity of the condition, but the blood is not in the ordinary sense of the word a tissue. It is a mixed secretion from the hæmopoietic organs, and for this reason the marrow naturally gives a clearer and more accurate picture than does the blood. For instance, changes indicative of an approaching relapse will be found in the marrow earlier than in the blood, and, further, as all forms of treatment of leukæmia are directed towards the regulation of marrow function, it is of great value to follow the effects of therapy by repeated sternal punctures. It should be recognised that whatever the improvement in the clinical condition the marrow is always recognisable as leukæmic. Emile-Weil and Perles have demonstrated this quite clearly by regular sternal puncture during the treatment of a case of myeloid leukæmia. The points to be looked for are variations in the relative percentages of the leukæmic cells, e.g., the increase in the number of myeloblasts foreshadowing the change from chronic to acute myeloid leukæmia.

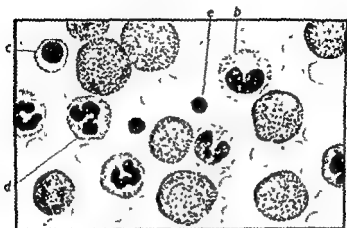
Chronic Myeloid Leukæmia (Chronic Leukæmic Myelosis) (Plate 4) The blood picture in this disease is usually striking and characteristic, but the changes in the marrow are less easily interpreted. After all the marrow is the main organ in which granular leucocytes are formed, and it is extremely difficult to recognise slight degrees of overgrowth. Fortunately, changes in the proportions of the cells occur early, and may enable one to make a diagnosis. Thus, in health, almost all the polymorphs arise from mitotic divisions of pre-existing myelocytes, myeloblasts are scanty and play little or no part in the production of fresh myelocytes. In severe infections, where the demand for granular leucocytes is greatly increased, there may be so great a strain that the myeloblasts not only increase in number but also add to the stock of myelocytes.

In chronic myeloid leukaemia, even in the stages when there is no increase in the number of cells in the blood, the myeloblasts take part in the formation of myelocytes, in other words, the whole process of granulopoiesis starts from a more primitive level. If, therefore, an excess of myeloblasts is found in marrow films, and if infection can be excluded, a diagnosis of leukaemia is very probable. When the leukaemic process is well established, the cellularity of the marrow is much increased, and this is so extreme that it can be recognised in well spread marrow-films where one may find as many as 80 cells in each field of the microscope. In the ordinary chronic stage of the disease, this increase depends almost entirely upon the addition to the number of myelocytes, which may form as much as 80 per cent of the white cells. Most of them are neutrophils, but excessive numbers of eosinophil and basophil myelocytes are also present. Almost all the myelocytes of whatever type are larger than the corresponding cells found in the blood, and many of them are less mature. It is particularly in the marrow that one can easily find myelocytes containing both eosinophilic and basophilic granules.

Mitotic figures are not numerous in chronic cases, but it is very rare to be unable to find a few, usually in myelocytes and much more rarely in non-granular cells, which are presumably myeloblasts or haemocytoblasts. The number of myeloblasts and of premyelocytes is distinctly greater than in health, and some prognostic inferences can be drawn from the number of these immature elements. The more plentiful they are the worse the outlook. In brief, it can be said that any great increase in the number of cells, less differentiated than myelocytes, is an indication either that a relapse is imminent or that the disease is passing from the chronic into the acute stage. This is, of course, comparable with what has long been known about the changes in the blood, viz. that the greater the percentage of immature cells the more likely is an acute exacerbation; but the marrow changes precede those in the blood and therefore permit of earlier treatment. On the other hand, undue alarm should not be aroused by some myeloblasts in marrow films. Even if none can be found in the blood some will be seen in the marrow in all cases, and, furthermore, they are always more numerous in the marrow than in the blood.

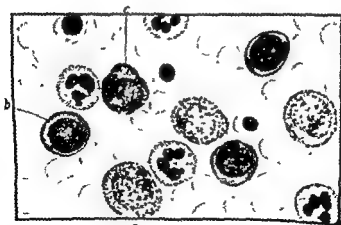
There are two other points of importance which are not easy to determine but which experience has shown to be of importance. The first is that the background of marrow films in chronic myeloid leukaemia is denser and contains more fragments of debris than in

PLATE 4



A CHRONIC MYELOID LEUKEMIA

- (a) Myelocyte (c) Basophilic normoblast
(b) Metamyelocyte (d) Polymorph
(e) Normoblast



B CHRONIC MYELOID LEUKEMIA BECOMING ACUTE

- (a) Myelocyte (b) Myeloblast (c) Basophilic Myelocyte

health. Recognition of this change naturally depends upon experience of marrow films in general. The second point, which is of great importance and which cannot be detected in films that are too thin or which are made from marrow fluid that is too dilute, is that the cells, or some of them, tend to lie in groups, in each of which there is only a single cell-type. Thus, it is quite common in fairly thick films to find islets of myelocytes, promyelocytes and myeloblasts, indicating that in leukæmic marrow the immature cells have a more focal arrangement than normal. It is in such islets that mitotic figures are most commonly seen. If such figures are present in the myelocyte islets, the outlook is much less grave than it is when the myeloblasts show many mitoses.

Marrow films in chronic myeloid leukæmia are the more pleomorphic because the leucoblastic overgrowth is accompanied by both erythroblastic and megakaryocytic reactions. This, of course, introduces a difficulty in interpretation, because, unless the marrow is very carefully studied, the fact that every type of cell is increased might lead one to suppose that one was in the presence of a *stern*-reactive hyperplasia such as may occur in association with any infection. The recognition of the focal arrangement of the immature white cells and of the increase in the number of myeloblasts is the main safeguard against this false inference.

It cannot be emphasised too strongly or too often that it is essential to correlate the blood picture with the myelogram, either alone can be most misleading. For instance, there is no peculiarity in the *bone* marrow that would enable one to recognise the fact that the *blood* picture was aleukæmic. The amount of hyperplasia is no greater in leukæmic cases than it is in aleukæmic ones, unless, of course, the aleukæmic state is due to treatment. The factor that regulates emigration of cells from the marrow into the blood is unknown. At this point it may be well to mention that examination of the *marrow* is of particular importance in those cases in which the *haematologist* has shown that the blood picture is aleukæmic. Such a state may be due to partial aplasia of the marrow, either idiopathic or as the result of excessively energetic treatment, or it may be due to some *other* cause which prevents emigration of cells from the *interstitial* marrow into the blood. It is obvious that in the former case any method of treatment such as X-rays will induce more rapid and more intense degeneration but, in the hyperplastic cases, irradiation of the marrow is as safe as in the ordinary leukæmic type of case.

Moeschlin has compared the effects of urethane on the marrow

in chronic leukæmia with those of X-rays and arsenic. He points out that the latter agents cause a diminution in the number of

the number of mitoses in the immature white-cells, while there is an increase in the number of dividing red cells. Thus, urethane acts by a selective inhibition of mitosis in the essential "neoplastic" cells of the leukæmic process. But the marrow does not show as great a decrease in the number of immature cells as it does after X-rays or arsenic.

There are other cryptic forms of myeloid leukæmia in which

æmic or even in

in these cases

leukæmia but in

tenable because the blood picture and clinical condition deteriorate until death occurs. Indeed, in prolonged cases, the medullary form of myelosis may develop into the ordinary type of chronic myeloid leukæmia. Early diagnosis by marrow puncture is of importance because radiotherapy causes improvement even at a time when the spleen is still unaffected.

There is also an acute form of this purely medullary overgrowth—*acute aleukæmic myelosis*—which clinically resembles the similar lymphatic condition.

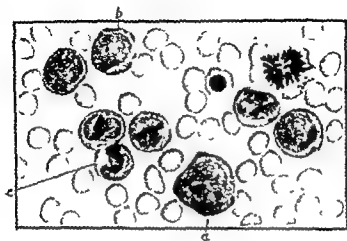
The relationship of *multiple myeloma* to *plasma-cell leukæmia* has not been clarified, but it is true to say that the more nearly *plasma-cell leukæmia* resembles typical leukæmia the more malignant is the process and the fewer are the nodular infiltrations.

In the marrow it is, as a rule, not possible to distinguish *plasma-cell leukæmia* from *plasma-cell myeloma*, but, if films show a monotonous series of plasma cells unmixed with other elements, one is probably dealing with the latter condition. The whole subject has been dealt with in some detail by Moss and Ackerman.

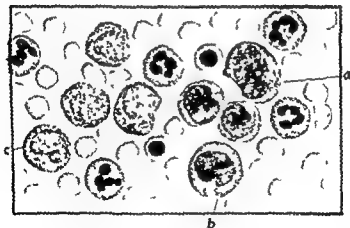
The rare condition known as *eosinophile leukæmia*, which may run either an acute or a chronic course, presents varying appearances in the bone marrow.

Acute cases usually end as *myeloblastic leukæmia*, but sometimes

PLATE 5



A MONOCYTIC LEUKEMIA
(a) Monoblast (b) Promonocyte (c) Monocyte



B MONOCYTIC PHASE IN MYELOID LEUKEMIA
(a) Monoblast (b) Monocyte (c) Myelocyte

the predominant cell in the blood and in the bone marrow is the eosinophile myelocyte and this may be the case even in association with a very acute course. In most of the chronic cases the majority of the eosinophiles in the circulation and in the bone marrow are of the mature type so that it is impossible from either blood or marrow examination to be certain whether the disease is chronic or acute.

A somewhat similar malady may occur in an acute or more rarely a sub acute form—the so called *aleukaemic myelosis*. This runs the clinical course of a rapidly progressive, severe anaemia without any change suggestive of leukaemia in the white cell count but the marrow has all the characters of the intensely active tissue found in ordinary cases of leukaemia. Strangely enough one finds that the predominant cells in the marrow are myelocytes not as one would expect in an acute case myeloblasts. If a case of this sort is encountered, it is extremely easy to draw false inferences concerning the acuteness of the disease if one is basing prognosis entirely on the characters of the myelogram. The disease is associated with a variable degree of anaemia which is often macrocytic although it may be normocytic or rarely, microcytic. When a macrocytic anaemia is present the marrow films will show a megaloblastic reaction and the picture simulates pernicious anaemia complicating leukaemia—the so-called leukaemia of Leube. Most modern writers regard the occurrence of these two diseases in one patient as fortuitous.

Myelosclerosis may be very difficult to distinguish from medullary myelosis without the aid of sternal puncture, as the blood counts and physical signs may be almost identical in both diseases. Most cases show fibrous tissue replacement of the marrow (which may lead to a dry puncture and the necessity for trephining (p. 87)) and no evidence of myeloid hyperplasia. As in medullary myelosis, the anaemia may be macrocytic, normocytic or microcytic, and the marrow will show the corresponding changes, in the last two types there are usually large numbers, up to 50 per cent of basophilic and polychromatic normoblasts.

Chronic Lymphatic Leukaemia (Chronic Lymphadenosis)
The myelogram in this disease is usually easy to interpret because lymphocytes are not numerous in normal marrow and rarely show any signs of mitotic activity. In advanced cases of chronic lymphatic leukaemia, marrow films are as monotonous as blood films. Lymphocytes are found so closely packed together that they almost resemble

lymphatic tissue which is interrupted here and there by a few small nests of granulocytes and red cells. The background of the marrow films is moderately dense but structureless and unlike that seen in chronic myeloid leukaemia where free cell granules are embedded in it. In almost every case the majority of the cells are small lymphocytes, but a variable number of larger forms can be found—not all of these are lymphoblasts, which, indeed, are usually scanty. They increase in number only if the disease is actively progressing or is about to become acute.

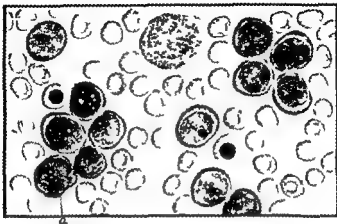
In less advanced cases of chronic lymphatic leukaemia, the lymphocytes can be seen to form small focal masses embedded in hyperplastic marrow tissue. In other words, the arrangement is similar to that in chronic myeloid leukaemia where we have already described foci of myeloblasts, etc. Thus, lymphocyte aggregations in the marrow never possess germ centres, and indeed, it is mainly at their periphery that the less immature forms are seen, and mitotic figures are found.

Chronic lymphatic leukaemia is notorious for its tendency to run an atypical course. Most cases of so called crypto leukaemia are of lymphatic type.

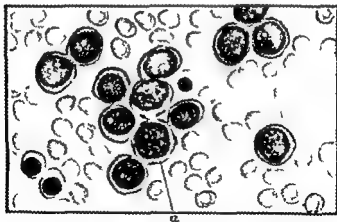
Chronic Aleukæmic Lymphatic Leukaemia This disease may run the whole of its course without any of the classical clinical signs of leukaemia. Quite frequently the picture is that of aplastic anaemia—great reduction of red corpuscles and of hæmoglobin, no signs of blood regeneration, leucopenia, and relative or absolute lymphocytosis. In the late stages, an occasional lymphocyte of immature type or even a lymphoblast may be found in the blood, but, in the past, diagnosis was often deferred until autopsy and was sometimes impossible until histological sections were examined. Now, sternal puncture permits of an accurate diagnosis of lymphadenosis, but the myelogram itself gives no indication that the blood picture is aleukæmic. The marrow changes are the same as in the blood.

... the blood is large or small
... but
... but
there seems to be much confusion in the literature as to what shall be called monocytic leukaemia, and what mixed myeloid and monocytic leukaemia. In our opinion the term, *monocytic leukaemia* (Schilling type, Plate 5A), should be limited to those cases which run an acute or sub acute course (never chronic), show a leucocytosis due to an increase in monocytes or their precursors, and an aplastic

PLATE 6



A ACUTE MYELOID LEUKEMIA
(a) Myeloblast (see p. 4)



B ACUTE LYMPHATIC LEUKEMIA
(a) Lymphoblast (see p. 6)

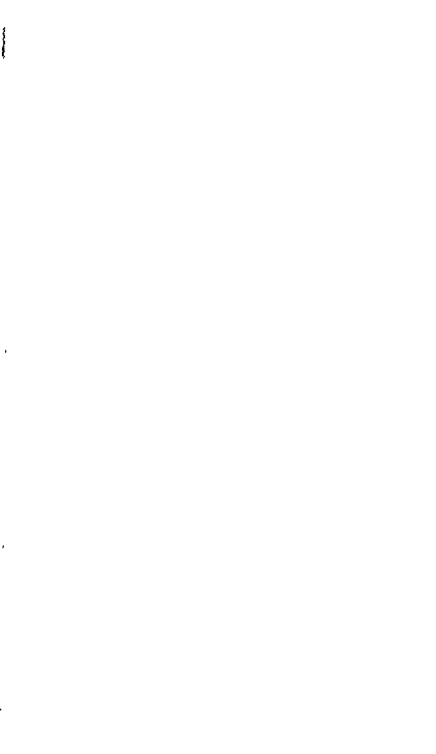
or hypoplastic type of anemia. The so-called mixed (Nægel, Plate 58) types are to be regarded as variants of myelogenous leukemia. In these, there occur transitory increases in the number of monocytic elements in the blood and the marrow which are probably atypical myeloblasts, but the general clinical and hæmatological picture, throughout the greater part of the illness, is identical with the myeloid type of leukemia. This is even more evident in tissues examined histologically, here the reticulo-endothelial system shows the changes characteristic of myeloid leukemia.

The marrow films in true monocytic leukemia are quite characteristic. As in all leukæmias there are many cells, monocytes and pro monocytes predominate and form from 70 to 90 per cent of all the nucleated cells. The mature monocytes have lobed or rounded nuclei with fine reticular chromatin, and a varying amount of cytoplasm which may or may not contain azurophilic granules. The monoblast is similar to, and may be difficult to distinguish from, myeloblasts and lymphoblasts although it commonly shows more variation in size than these cells. The promonocyte has a rounded nucleus without nucleoli and may show some azurophilic granules in the cytoplasm. The number of mitotic figures is striking (many may even be found in the peripheral blood) and these are chiefly in the pro-monocytes. The number of hæmocytoblasts is increased and, indeed, these cells may be closely simulate from the promonocyte if the latter is at all atypical, and in monocytic leukemia it often is atypical.

Erythropoiesis is markedly depressed. There is a reduction of all the types of normoblast, but particularly of the earlier and more basophilic forms. Megakaryocytes and platelets are scanty. From films it is difficult to say whether the granulocytic cells are affected, but quite often the absolute number of neutrophile polymorphs in the peripheral blood is little, if at all, reduced.

In the "monocytic" phase of myeloid leukemia atypical myeloblasts resembling monocytes appear in varying numbers in the blood and marrow. As this phase only occurs in the chronic form of myelogenous leukemia, an erythroblastic hyperplasia of the bone-marrow is to be expected, unless, of course, the malady is in its terminal stages and aplasia of the erythroblastic tissue has occurred.

Acute Leukæmia (Plate 6) This disease may be primary or it may be the terminal phase in a chronic case. It is usual to distinguish between acute myeloid and acute lymphatic leukemia, although the differentiation is of purely academic value. The



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Acute Leukæmia (Plate 6). This disease may be primary or it may be the terminal phase in a chronic case. It is usual to distinguish between acute myeloid and acute lymphatic leukæmia although the differentiation is of purely academic value. The

affects the erythropoietic tissue, there is less difficulty than when the leucoblastic cells are involved. In the latter case, it may be difficult to differentiate simple reactions from leukæmia, and, indeed, blood examination is often more important for diagnosis than is marrow puncture. It is only if the small focal masses of cells typical of leukæmia are found that a definite conclusion can be reached.

Other significant points have to be taken into account. Thus, if the marrow is found to be normal, the diagnosis of leukæmia is excluded. . . .

Even so, the histological arrangement of the cells is different. In infections a definite focal distribution is never found. Then also, the characters of the more mature cells may help to distinguish a leukæmoid reaction from genuine leukæmia, in the latter they may be abnormal in the sense of being malformed, but they never show the degenerative changes that are so common in infective states.

There can be no doubt that there is still need for much investigation of the marrow in the diseases that show a leukæmoid blood picture, and at the present time we are not in a position to say more about them.

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NEOPLASTIC AND ALLIED CONDITIONS OF THE BONE-MARROW

IN so far as they directly affect the myelogram, bone-marrow tumours fall into two groups. In the first are the *generalised neoplasms of the hæmopoietic tissue* where sternal puncture may show the marrow to consist almost entirely of tumour cells. In the second, the tumours are *localised* and unless a nodule of growth is punctured, the myelogram shows only the effects produced by compression, replacement and irritation, *i.e.*, leuco erythroblastosis, which may progress to hypoplasia. In this group, the tumours may be primary in the bone marrow or metastatic from growths elsewhere in the body, and it must be borne in mind that it is only when this type is fairly widespread in the marrow that hæmic erythroblastosis is produced. Tumour cells will not be found in marrow films unless a nodule is present at the site of puncture.

In our present state of knowledge any attempt to classify bone-marrow tumours must be tentative and somewhat controversial. As, in this book, we are concerned chiefly with marrow puncture as a method of clinical and cytological investigation, most of the discussion will be devoted to the most helpful methods of elucidating the myelogram, but the possible relationships of the several conditions must first be considered.

Bone marrow is mesenchymal in origin and most of the elements of the reticulo-endothelial system are represented in it, so that a variety of neoplastic conditions may arise as primary "tumours," including the reticulososes, reticulo sarcomata and the myelomata. The pathology of the reticulososes has been put on a sound basis by Robb Smith in this country and Gall and Mallory in America. Both classifications are cytological, the authors all agreeing that gross anatomical and clinical classifications have no practical value. Gall and Mallory classify their cases according to the degree of development of the cells involved, while Robb Smith bases his arrangement on anatomical grounds. Thus we have the *follicular reticulososes*, the parent cell of which is the reticulum cell of the germinal follicle of the lymph-nodes, the *sinus reticulososes*, which arise from the living cells of the sinuses of the reticulo-

endothelial system, and the *medullary reticulosos*, which take origin from the free reticulum cells or their descendants, lying in the stroma of the reticulo-endothelial tissue between the sinuses. In the last group there are lymphoid, myeloid and monocytic medullary reticulosos, which we commonly call lymphatic, myeloid and monocytic leukaemia respectively. This group also contains the metabolic reticulosos of Gaucher's disease, Niemann-Pick disease and xanthomatosis. *Fibro-myeloid medullary reticulosos*, or Hodgkin's disease, is yet another component of this group.

The *reticulo-sarcomata*, including lymphosarcoma, should not be regarded as a separate group of diseases but as a more malignant phase of the corresponding cell type reticulosis (Table II).

TABLE II
RELATIONSHIP OF EWING'S TUMOUR AND MYELOMATA TO
RETICULO-ENDOTHELIUM OF BONE-MARROW

BONE MARROW CELL	RETICULOSIS	MALIGNANT TYPE
MESENCHYMAL CELL	RETICULUM CELL RETICULOSIS	(EWING'S TUMOUR)
HEMOCYTOBLAST		RETICULUM CELL MYELOMA HEMOCYTOBLASTIC MYELOMA
ERYTHROBLAST	POLYCYTHAEMIA VERA	ERYTHROBLASTIC MYELOMA
MYELOBLAST	MYELOID LEUKAEMIA	MYELOBLASTIC MYELOMA
PLASMOBLAST	PLASMA CELL LEUKAEMIA	PLASMA CELL MYELOMA

The myelomata form a group of tumours which arise from blood cells or their precursors. The hemocytoblast, a direct descendant of the primitive mesenchymal cell, is now commonly supposed to be the parent cell of all blood cells and from it arise the blast cells which differentiate to the adult red corpuscle, granulocytic, monocyte, lymphocytic and plasma cell. This line of development is shown in Table III.

Theoretically any of these groups of cells may produce tumours, and hence myelomata, but in practice the term is reserved for these tumours which occur primarily in the bone-marrow. Lymphatic and monocytic tumours occur in the lymphadenoid tissue rather than the marrow and should be considered with the reticulosos. It may be said that plasma cells also are mainly produced in the lymphoid organs, but they also occur in the blood-forming bone-

marrow and glands of cases of acute and chronic myeloid leukaemia. The chief significance of the disease is that it presents a typical leukaemic infiltration of the bone marrow and viscera with a leukaemic blood picture and at the same time produces bulky medullary tumours which provide all the essential features of a malignant neoplasm, that is, active proliferation atypical cells, distant metastases, some of which are clearly of embolic origin, and destructive local infiltration. In fact, the disease is a mixture of leukaemia and malignant myeloma.

In another group of cases with radiologically typical myelomata a leukaemic or subleukaemic blood picture is present. In fact, Pincus and Riach have described four grades of plasma cell myeloma depending on the type and extent of bone involvement and the condition of the blood. These are —

- (1) Much bone destruction and no blood changes
- (2) Moderate bone destruction and a few plasma cells circulating in the blood stream
- (3) Bone destruction less with a diffuse marrow infiltration and a larger number of circulating plasma cells
- (4) Diffuse bone-marrow infiltration without erosion and a frankly leukaemic picture in the blood and viscera. This is plasma cell leukaemia.

A third group of cases which appear intermediate between the two conditions are those whose peripheral blood picture and sternal marrow are typical of leukaemia but there is no enlargement of the spleen or liver. In these, X-ray of the long bones will often reveal several small areas of rarefaction with loss of bone pattern, suggesting a mild degree of erosion by local deposits. Fig 11 is an X-ray of the hand of one such case. The patient was a girl aged about eight years old who was originally seen complaining of pain in the hands and feet. Complete radiological examination of the skeleton showed only the lesions in the figure and similar areas of rarefaction in the 5th metatarsal bones. The diagnosis of leukaemia was confirmed by blood count and marrow puncture. The girl died at home within a year and no post mortem examination was possible.

These intermediate types of case form a definite link between leukaemia and multiple myeloma and are probably more common than is generally realised. Many conditions labelled simply leukaemia are atypical when closely studied and the deviations from the classical picture are often towards the myelomatous syndrome.

FIG. II



EROSION OF BONE IN LEUKEMIA

Ewing's tumour, or endothelioma of bone, must also be considered in any discussion of myelomata. In this disease, tumours growing within the marrow cavities of the bones cause irregular absorption of the shaft and widening of the marrow cavity. In the later stages the bone may be completely destroyed. The regional lymph nodes are often involved and pulmonary metastases occur in nearly all cases. The clinical course of the disease is more rapid than with myeloma and the tumours are more invasive.

Ewing himself thinks the tumour arises from the endothelium of the blood vessels of the bone marrow, but other authors, notably Oberling, claim to have observed differentiation of the tumour cells into plasma cells, myelocytes and even primitive red cells. If this is so the tumour may well be an *haemocytoblastoma* or *myeloma* of the most primitive blood cell, especially as these tumours commonly occur in the ends of long bones in children and infants—that is during the age period when these bones contain haemopoietic marrow.

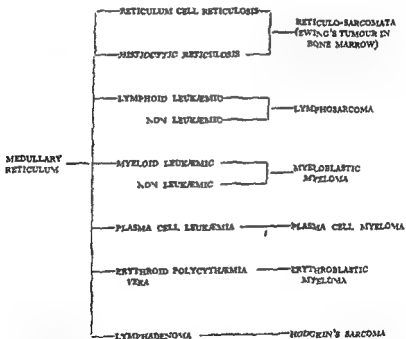
As the haemocytoblast is a direct descendant of the reticulum cell and mesenchymal cell, which also may form the endothelial lining of blood vessels, a reticulum cell myeloma in the bone marrow may well show angioblastic tendencies in some cases, thus accounting for the features stressed by Ewing. As also the primitive mesenchyme is situated perivascularly, they may be included in the "perivascular endothelium," which Ewing cites as the possible origin of these tumours. In fact, the implications of a multipotent primitive cell in the neoplastic process is the only way to account for the varying ways in which these tumours tend to differentiate.

Those tumours of bone-marrow which are included in the group known as Ewing's tumour may therefore be classed as myelomata of the more primitive cells of the reticulo-endothelial system in the bone marrow and any of them may tend to show differentiation along the lines of the more adult bone marrow cells. The greater clinical malignancy of these tumours is accounted for by the more primitive nature of the cells involved. This group may be regarded as the sarcomatous lesion of these primitive reticulo-endothelial cells as opposed to the systematised overgrowth known as reticulum cell reticulosis, which in the bone-marrow is represented by the multiple endotheliomas of bone first described by Marckwald. The cells are the same clear polyhedral cells as seen in Ewing's tumour, and although death occurs in two years the tumour does not show the usual features of malignancy like Ewing's tumour.

The patients die of anæmia and cachexia with severe pyrexia—a type of illness which compares closely with the condition seen in animals when the RES is disorganised by blockage with dye, etc

In the bone-marrow, therefore, we can demonstrate a relationship of the different tumours in the same way as can be demonstrated in the rest of the reticulo-endothelial system. This relationship is shown in Table V. Table IV shows a more complete classification, including the other reticuloses and their sarcomatous manifestations

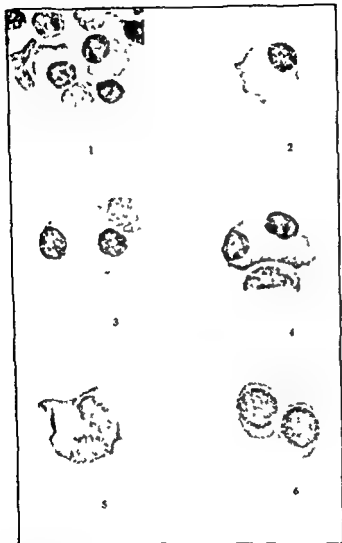
TABLE V
HYPERPLASTIC AND SARCOMATOUS RETICULOSES



Myeloma. There are four generally recognised types of myeloma —

- (i) plasma cell,
- (ii) myeloblastic, derived from the primitive myeloid cells,
- (iii) erythroblastic, derived from the nucleated red cells;
- (iv) lymphoblastic, derived from the lymphoid cell series, and which is probably secondary to lympho sarcoma elsewhere

PLATE 7



PLASMA CELLS FROM CASE OF PLASMA-CELL MYELOMA

- | | |
|------------------------------------|--|
| 1 Plasmoblasts | 4 Binucleate plasma cell |
| 2 Mitotic division of plasmoblasts | 5 Normal plasma cells |
| 3 Binucleate plasma cell | 6 Plasma cell with cytoplasmic vacuola |

The majority of myelomata are composed of elements resembling plasma cells; indeed, there is little reason for doubting that they are really plasma cells. But, according to one's opinion, these tumours would equally properly be designated as pseudo plasmacytomata.

We have already seen that the nature of plasma cells is still uncertain (p. 6), though that need not affect our descriptive knowledge of tumours composed of them, but it must be emphasised that it is often possible to observe what appears to be a continuous series of stages between the cells of the reticulo-endothelium and typical plasma cells. But, of course, these elements, as seen in

which is vacuolated round the nucleus (*heller Hof*). In films, there is much more variation in size, some of the cells being no larger than small lymphocytes, whilst others are as large as monocytes, and may (in marrow but not in blood) contain more than one nucleus. The basic chromatin is much more variable in its distribution than it is in the plasma cells of the tissues: it may be "cart-wheel," but it is often quite irregular; similarly, it may be symmetrically or excentrically placed in the nucleus. The cytoplasm is basophilic, but less intensely so than in the tissues: it may be vacuolated but not necessarily in the vicinity of the nucleus, and occasionally it is seen to contain azurophilic granules such as rarely occur in tissue plasma cells. Mitotic figures are often observable. Azurophilic protein crystals may be found in a few "myeloma cells" (Steinmann).

Marrow films, thus, present a very varied appearance, which is extremely suggestive of neoplasia, but which does not always immediately suggest that the predominant element is the plasma cell. But, even so, a little experience allows a diagnosis of myeloma to be made with much greater certainty than is possible even with X-rays (Rosenthal and Vogel). Of course, the film does not consist entirely of plasmacytoid elements: it is very rare for all the ordinary marrow cells to be replaced. As a rule about 50 per cent of the cells are of plasma cell type. But, if the needle has penetrated a nodule of "tumour," it may be found that almost every cell is of this kind. But, from the point of view of diagnosis, these differences are of no special significance.

In the *myeloblastoma*, the cells are, on the whole, larger and may contain few or many neutrophilic granules in the cytoplasm. Many

polymorphonuclears and myelocytes are present, and both may be found in the peripheral blood

In the *erythroblastoma*, the normoblasts are usually well hæmoglobinised, but there may be a large percentage with basophilic cytoplasm. The cells are small, and the nuclei pyknotic. It is still uncertain whether erythroblastic tumours, in which there are no hæmoglobinised elements, really exist

Snapper and his co-workers have shown that treatment of multiple myeloma with stilbamidine often results in the development of peculiar changes in the plasma cells in the marrow. These take the form of basophilic granules in the cytoplasm. Similar changes occur after treatment with antimony as a specific result of the effect on ribose nucleic acid in which the cytoplasm of myeloma cells is rich. It would appear, therefore, that the nucleoprotein of myeloma cells differs from that of other cells which do not react in this way with antimony or stilbamidine

Chloroma As a rule, symptoms and signs suggestive of acute or sub-acute leukæmia precede the development of chloromatous tumours, but, occasionally, there are no changes in the blood, except perhaps slight hypochromic anæmia, even at a time when quite large masses are present in the orbits. Then, examination of the marrow is likely to be very helpful, especially as in this disease, mitotic activity is remarkably intense. The tumour cells are scattered throughout the marrow, some being isolated, others in groups of six or nine, and the adjacent myeloid tissue is distinctly hyperplastic. Later, when the tumour has spread, the greater part of the marrow may be replaced by abnormal cells, and it is then difficult to discover any remnants of normal tissue

The characteristic cells are of the same type in every case of chloroma. They are large, with a bulky nucleus, which contains distinct nucleoli. The basi-chromatin has an almost reticular distribution, and the cytoplasm, which is moderately basophilic, contains no granules. Cells identical with those in the marrow are only rarely found in the circulation, where the predominant cells are usually myeloblasts or extremely immature premyelocytes. It is this latter fact that suggests that all cases of chloroma are of myeloid nature, although some writers still contend that lymphatic examples do occur. It is, however, more probable that chloroma represents a state intermediate between true acute leukæmia and a neoplastic condition

Reticulo-Endotheliosis. The *follicular reticulososes* produce no

change in the peripheral blood picture, and it is only when secondary nodules develop in the bone marrow that we get a picture of leucoblastic and erythroblastic hyperplasia, which may eventually develop into aplasia of the marrow if the invasion by tumour becomes more or less complete.

The *sinus reticulosos* also show no abnormality in the peripheral blood, apart from moderate anaemia and slight thrombocytopenia, but they do cause marked changes in the bone-marrow. For this reason, Dameshek, who has reviewed most of the recorded cases, refers to them as *aleukæmic sinus reticulosos*. The bone-marrow presents a varied picture. Characteristically, there are small collections of large cells, 20 to 30 microns in diameter, each with a rounded or indented nucleus, which occupies more than half of the

of the cells show azurophilic granules in the "Hof" of the nucleus. Mitotic figures are frequently seen. The myeloid and erythroid elements are decreased, and megakaryocytes are absent. This probably represents a hypoplastic or destructive condition of the bone-marrow, and it is interesting to note that in none of the recorded cases has there been an initial hyperplasia. It seems a reasonable inference that the tumour cells are not merely replacing the normal marrow elements, but that they arise from a primitive cell, which is also the precursor of the blood cells. Consequently, on account of this perversion of function, the hæmopoietic tissue is unable to react in the ordinary way.

Of the *medullary reticulosos*, the leukæmias are discussed elsewhere (p. 15).

Gaucher's disease usually affects the spleen more intensely than the bones, although skeletal affection may occasionally predominate. But, even in the clinically splenomegalic cases, some involvement of the bone-marrow is common. How usual it is was not realised until sternal puncture became a fairly common procedure. This frequency of skeletal involvement is an important matter, because it has been suggested that splenectomy, in such a metabolic disease as this, would accelerate the deposition of the Gaucher substance in the bones, so that the last state might well be worse than the first. Logan has shown that the fear is unfounded and that there is, in fact, improvement in almost every case after removal of the spleen.

The Gaucher cells in the marrow are large (50 microns or more).

The nuclei present no characteristic arrangement of the chromatin, but are always relatively small and dark, several may be found in one cell. The cytoplasm is bulky, with sharply defined outlines. Some of the cells are polygonal, others elongated, but few transitional forms are seen. In paraffin sections, the cytoplasm has a foamy appearance, due to solution of the contained lipid material, and a faint, diffuse, iron reaction can often be obtained. There seem to be three fairly distinct stages in the development of these elements. Thus, the least differentiated cells have granular cytoplasm, in which lies a more or less reticular nucleus. Then, there are cells with similar cytoplasm, but dark and almost structureless nuclei, and lastly, the fully developed Gaucher cell, with vacuolated cytoplasm and small dense nucleus. It is only in the last type that it is possible to detect the presence of fine fibrils, which are best shown by silver-impregnation methods.

The myeloid tissue itself is usually hyperplastic, in contrast to the peripheral leucopenia, and there is a slight increase of monocytes (up to 10 per cent) in the myelogram. This is not sufficient by itself to arouse a suspicion of Gaucher's disease, but it does give support to the view that monocytes may arise from the same primitive reticulum cell.

Niemann Pick disease shows a similar invasion of the marrow with "tumour" cells, and hyperplasia of the myeloid tissue. In contrast to Gaucher's disease, however, there is usually a peripheral leucocytosis. The characteristic cells are large (20 to 80 microns) and contain a reticular nucleus. The cytoplasm is filled with droplets that give rise to a "foamy" appearance very different from the fibrillary appearance of the typical Gaucher cells. As the disease is always fatal by the age of two years, marrow biopsy must be done by tibial trephine, or smears must be made from spleen puncture.

Hodgkin's disease affects lymphatic glands so much more obviously than it does the marrow, that relatively little attention has been paid to the latter tissue. And, although we probably never obtain assistance in diagnosis by examining the bone marrow, there are points of interest and importance in connection with it.

The cases fall into four groups, viz., those with normal marrow, those with *leucemia*, those with *lymphadenoma*, and those with *lymphadenoma*.

In the third group it is not, as a rule, possible to be certain that

dyshæmopoietic In the following chapters the main changes from

in the marrow

Hæmorrhagic Anæmia . Anæmia following hæmorrhage may be acute or chronic, depending on the amount, frequency, and duration of the bleeding. Acute anæmia is most commonly seen following trauma, bleeding from a peptic ulcer, or childbirth. Providing that treatment is adequate, the blood loss is replaced in about thirty days and this replacement is brought about by an increase in the normal activity of the bone marrow.

The chronic type, usually induced by long continued, frequent and relatively small bleedings, such as occur in menorrhagia and some types of peptic ulcer, produces characteristic changes in the myelogram. A study of these two types of marrow change helps us to interpret the findings in hæmorrhagic anæmias due to abnormalities of the blood or blood vessels, of which thrombocytopenic purpura and hæmophilia are examples. The special findings peculiar to these anæmias will be discussed later. Certainly the myelogram in traumatic anæmias does not help in diagnosis, either of the nature of the anæmia (which is usually obvious on clinical grounds) or of the site of the loss of blood.

Acute hæmorrhagic anæmia The myelogram here shows increased normal activity. Both the erythroblastic and myeloid elements are affected, the former more than the latter. The hyperplasia of the myeloid tissue is reflected in the peripheral leucocytosis.

The normoblasts are increased in number and show a tendency to become hæmoglobinised at an earlier stage, i.e., the usual basophilic normoblast becomes polychromatic, and the polychromatic one becomes eosinophilic. The number of reticulocytes present is markedly increased. Megakaryocytes are more frequent and may be seen in moderately large numbers in the thick end of the film. In the percentage count the polymorphs are decreased, but this decrease is only apparent because the total number of cells is increased. Mitoses are more numerous, indicating general hyperplasia. The mitoses, both of red and white cells, takes place at the normal level of cell development. The myelogram is as follows —

Neutrophiles

Myelocytes

Metamyelocytes

30-35 per cent

10-15 " "

Eosinophiles		
Myelocytes	.	1-2 per cent.
Polymorphs	.	1-2 " "
Basophiles		
Myelocytes	.	3-6 " "
Polymorphs	.	1-2 " "
Premyelocytes		1-2 " "
Hæmocyto blasts		2-4 " "
(including myeloblasts)		
Lymphocytes		6-12 " "
Pro-erythroblasts		1-2 " "
Normoblasts		25-30 " "
Megakaryocytes	.	frequent

Chronic Post hæmorrhagic Anæmia When the anæmia is but moderate the bone marrow activity is only slightly increased. In more severe cases there may be a great increase in the amount of red marrow. This hyperplasia may eventually be succeeded by exhaustion, and the marrow then becomes hypoplastic or aplastic. In a fully reacting case, *s.e.*, before marrow exhaustion supervenes,

numerous mitotic figures of normal type. The myelocytes are little, if at all, reduced in number, and the total number of cells is somewhat increased. Reticulocytes are fairly numerous though not as abundant

that iron is needed for complete maturation of the cells, and not as a stimulus to erythropoiesis itself.

Neutrophiles		
Myelocytes	.	20-30 per cent
Metamyelocytes		5-8 " "
Polymorphs		8-12 " "
Eosinophiles		
Myelocytes		1-2 " "
Polymorphs		1-2 " "
Basophiles		
Myelocytes		$\frac{1}{2}$ -3 " "
Polymorphs		$\frac{1}{2}$ -3 " "

Premyelocytes	1 - 3 per cent
Myeloblasts	0 - 2 " "
Pro-erythroblasts	3 - 5 " "
Normoblasts	40 - 50 , "
Megakaryocytes	moderate

Hæmorrhagic Anæmia The specific marrow changes in *essential thrombocytopenic purpura* will be described in the chapter on aplasia of the bone-marrow

In true *hæmophilia*, apart from the changes described above, the marrow films show a marked increase in the number of megakaryoblasts and megakaryocytes, but the number of platelets is within normal limits. Custer and Krumbhaar have described the appearance of the bone-marrow in three fatal cases. Lumarzi, Poucher and Birch have shown that sternal puncture can be safely performed on hæmophiliacs and they confirm the findings described.

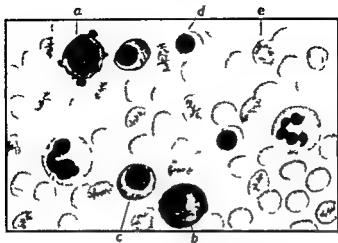
Hæmolytic Anæmia (Plate 8A) In all forms of hæmolytic anæmia the activity of the marrow is at its maximum. There is great and continuous normoblastosis which is presumably induced by loss of corpuscles, due to destruction in the body, but whether any erythropoietic stimulus is liberated by the destroyed cells is still uncertain, although probable.

The clinical state is not clearly, if at all, reflected in the marrow, which is in a condition of extreme activity throughout the course of the *malady*. About three quarters of the cells in the marrow are nucleated red-cells in various stages of development, some are mature, but the majority are basophilic and possess large cart-wheel nuclei. Another striking feature is the presence of considerable numbers of cells as immature as the pro erythroblast. These, as previously described, differ from megaloblasts but are at least as large, if not larger. In some cases a true megaloblastic reaction may develop as described by Farley in cases of Bartonella fever. The large number of reticulocytes is another characteristic feature.

The myelogram is of the type shown below —

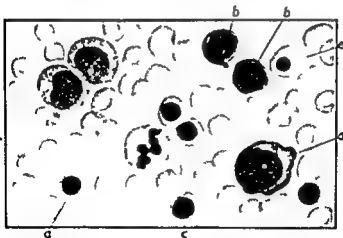
Neutrophiles	
Myelocytes	10-17 per cent
Metamyelocytes	2-5 " "
Polymorphs	2-5 , "
Eosinophiles	
Myelocytes	0-2 , ,
Polymorphs	2-5 "

PLATE 8



A HEMATOLOGIC ANEMIA

- | | |
|----------------------|---------------------------|
| (a) Pro-erythroblast | (c) Basophilic normoblast |
| (b) Plasma cell | (d) Normoblast |
| (e) Reticulocyte | |



B LEUKEMIA

- | | |
|---------------------------|--------------------------------|
| (a) Promegaloblast | (d) Normoblast |
| (b) Late normoblast | (e) Eosinophilic megakaryocyte |
| (c) Basophilic normoblast | (f) Megakaryocyte |

Lymphocytes	1- 2 per cent
Monocytes	1- 2 " "
Premyelocytes	5-10 " "
Hæmohistioblasts	1- 3 " "
Hæmocytoblasts	1- 4 " "
Pro-erythroblasts	25-34 " "
Normoblasts	20-30 " "
Megaloblasts	0- 2 " "
Megakaryocytes	not increased
Reticulocytes numerous May be as high as 80 per cent of the non-nucleated elements of the films	

In many of the hæmolytic anæmias the anæmia may be macrocytic in type. In all the groups one or more such cases are described in the literature. Apart from the true megaloblastic reaction in Bartonella fever, however, the characteristic feature of these marrow films is the high percentage of pro-erythroblasts. Limarzi has shown that an intense normoblastosis occurs in the marrow in the rare hæmolytic anæmia following sulphonamide poisoning and is accompanied by a macrocytic anæmia. The macrocytosis is, therefore, probably due to rapid maturation of the pro-erythroblasts and basophil normoblasts, the nucleus being extruded rather early in the normoblastic cycle. That it is not due to megaloblastosis is borne out by the fact that liver preparations do not cause improvement.

In the *infective group of hæmolytic anæmias*, the myelogram does not show such a high proportion of pro-erythroblasts, and the regeneration of red cells is not as a rule so intense. The hæmolysis is probably due to the infecting organism causing a "toxæmia" of the red cells which render them more liable to the normal process of destruction. This toxic action is also exerted on the bone marrow, which cannot then respond so rapidly. Where the infection is prolonged and the toxæmia extreme, the marrow fails to respond at all and becomes aplastic (*erythronoelasia*). We have recently seen a case of this type associated with tuberculosis, and sections of the bone-marrow removed at autopsy showed a few minute tubercles.

Gas gangrene infection (*Cl. Welchii*), and sometimes an anaerobic hæmolytic streptococcus, produce a hæmolytic anæmia in a different way. They produce a hæmolysis which acts directly on the red cells. In these cases, until the toxæmia becomes severe, the marrow may be expected to react more exuberantly.

Malaria produces a chronic hæmolytic anæmia, the hæmolysis being caused by direct action of the parasite on the red corpuscles. Even in malignant tertian infection, where the hæmolysis may be so intense as to produce blackwater fever, and early death, the marrow shows a well marked erythroblastic hyperplasia with large, intensely basophilic, normoblastic cells (see Chapter VIII).

Some hæmolytic anæmias are due to poisons—lead, potassium chlorate, benzedrine, phenylhydrazine, phosphorus, TNT, dinitrobenzene, etc. The reaction of the bone marrow to all these compounds is as that described for hæmolytic anæmias in general. Aplasia develops rarely, if ever, because the patient dies of some other toxic effect of the poison before the aplastic stage is reached, although a small number of workers with trinitrotoluene have died of aplastic anæmia. The sulphonamide group of drugs, however, may produce a hæmolytic anæmia, and, if still more is given, the marrow may become aplastic.

In lead poisoning, of course, the characteristic basophilic stippling of the red cells will be seen in marrow films, and Henning and Keilhack have reported ten cases where the stippling was found in the red cells of the marrow when absent in the peripheral blood.

The hæmolysis produced by incompatible transfusions and paroxysmal hæmoglobinuria causes no peculiar abnormalities in the bone-marrow.

In *acholuric jaundice*, in both the congenital and acquired type, no new information about the pathology of the disease has been acquired by studying bone-marrow films. The spherocytosis can be seen as easily as in the peripheral blood. A constantly high reticulocyte count may be found in the marrow even when the red cell count is nearly normal. The reticulocyte count may be normal during a remission of the disease and the spherocytosis may temporarily diminish. Spherocytosis is not so marked in the acquired form of the disease.

It has always been assumed that the crises that occur in congenital hæmolytic jaundice are due to sudden great increase of destruction of the red cells. This view has recently been challenged by Owen, who alleges that the rapid increase of anæmia is probably due to a temporary hypoplasia of the bone-marrow. And he has shown that the marrow changes at the onset of such a crisis strongly support this view.

Just before the crisis, the marrow shows the usual intense erythropoiesis of the disease, but within two or three days there

are practically no erythroblasts remaining in the marrow, then, as the crisis dies down, there is regeneration of pro-erythroblasts which mature into macroblasts, until, at the end of the crisis, the marrow shows an enormous increase of normoblasts, while at the same time, the number of reticulocytes in the circulating blood rises sharply. Owen's views, although not yet confirmed, would explain the low percentage or absence of reticulocytes that has long been known to occur during crises.

In *sickle cell anemia*, the latent phase is much more common than the active one. In the latent form, sickling of the cells is not found in the peripheral blood but has been found in the bone-marrow of a case in this phase coming to autopsy. The presence of sickle cells is not diagnostic of anemia, as the sickle cell trait has been found in 5.7 per cent. of all negroes, whether or not they are in good health. The phenomenon of sickling cannot ordinarily be seen in stained films. A fresh preparation sealed under a cover slip should be examined a few hours after it is made. The myelogram does not differ from that of any other form of hæmolytic anemia except, of course, in the shape of the red corpuscles. Perhaps, however, the number of monocytes containing red corpuscles or pigment is rather greater, and Wintrobe has recorded the presence of long bands of erythrocyte cytoplasm (about 2 microns thick) lying in marrow films.

In *Lederer's anemia* the marrow picture may be complicated by the presence of a leukæmoid reaction. This is usually myeloid in type, but a case has been described with the appearances of lymphatic leukemia in the peripheral blood, including lymphoblasts.

In *icterus gravis* the marrow changes are the same as in any hæmolytic anemia. Marrow puncture is not of much value because the peripheral blood reflects the condition of the marrow in showing a variety of normoblastic cells which leave no doubt as to the diagnosis. Besides, sternal puncture and tibial trephine are not suitable procedures to be employed on new-born babies.

Toxic Anæmias. This group contains the hypoplastic and aplastic anæmias. It is a rather heterogeneous group of maladies, which may be primary or secondary. If of the latter type, the cause may be poisoning of various kinds, infection or toxæmia. But, whatever the cause, the malady is a grave and dangerous one, and, in all, the marrow picture is similar, no indication of the cause can be inferred from the myelogram. The conditions are more described later with other aplastic conditions of the bone marrow.

Dyshæmopoietic Anæmias. The hæmopoietic bone-marrow is a complicated and highly specialised structure which has to supply the formed elements of the blood continuously and rapidly. In order that it may do this efficiently, an adequate and properly balanced supply of nutriment is necessary. This has long been recognised, but our knowledge of the food requirements of the blood-forming tissue has, until the end of the first quarter of this century, been confined to one constituent, iron. Now we know that the hæmopoietic principle, copper and other metals, thyroxin, and vitamin C all play their part. Lack of, or failure to utilise, any of these factors may result in anæmia. As a result of malnutrition of the bone marrow, two main groups of anæmia are produced (1) hypochromic and microcytic, (2) hyperchromic and megalocytic. The first group arises with deficiency of iron, internal secretion of thyroid, and vitamin C. The second arises with deficiency of the hæmopoietic principle.

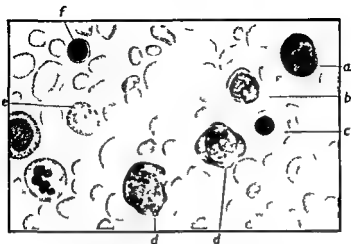
Deficiency of hæmopoietic principle —

Pernicious anæmia (Plates 9, 10 and 11) is, of course, the best known anæmia of this group. An exactly similar condition of the blood and marrow may obtain in other anæmias of the same type, whether the cause be carcinoma of the stomach, complete gastrectomy or other conditions giving rise to deficiency of the hæmopoietic principle.

The myelogram in pernicious anæmia is striking and characteristic. The constant feature is the presence of megaloblasts, but it is rather on the large number of these cells than on their simple presence that diagnosis is to be based. As mentioned earlier, some writers aver that a few of these cells can be found, even in health, it is, therefore, an increase that is significant. But there is another point even if the megaloblast be not regarded as peculiar to pernicious anæmia, there is no doubt that the marrow contains a larger number and far more young forms in this disease than are found in any other. It must not, however, be supposed that the whole of the erythropoietic process in this disease is megaloblastic in type, there is great normoblastic activity also. In other words, there is a mixed erythroblastic reaction of an intensity never seen in other maladies.

Little is known of the changes in very early cases, but Segerdahl states that the characteristic blood picture can be found before there are any recognisable changes in the marrow. But in definite, and untreated cases, the myelogram is very striking, inasmuch as there are two changes: intense megaloblastosis which of course, is

PLATE 9



A PERNICIOUS ANEMIA (UNTREATED)

- | | |
|----------------------------|------------------------------|
| (a) Early megaloblast | (d) Intermediate megaloblast |
| (b) Late megaloblast | (e) Stippled megalocyte |
| (c) Pernicious megaloblast | (f) Lymphocyte |

Note — Megalocytes, poikilocytes and anisocytes



B PERNICIOUS ANEMIA (EARLY STAGE OF TREATMENT)

- | | |
|-----------------------------|------------------------------|
| (a) Intermediate normoblast | (c) Intermediate megaloblast |
| (b) Early normoblasts | (d) Reticulocyte |

Note — Megalocytes less marked than in A

Dyshæmopoietic Anæmias. The hæmopoietic bone-marrow is a complicated and highly specialised structure which has to supply the formed elements of the blood continuously and rapidly. In order that it may do this efficiently, an adequate and properly balanced supply of nutriment is necessary. This has long been recognised, but our knowledge of the food requirements of the blood-forming tissue has, until the end of the first quarter of this century, been confined to one constituent, iron. Now we know that the hæmopoietic principle, copper and other metals, thyroxin, and vitamin C all play their part. Lack of, or failure to utilise, any of these factors may result in anæmia. As a result of malnutrition of the bone marrow, two main groups of anæmia are produced (1) hypochromic and microcytic, (2) hyperchromic and megalocytic. The first group arises with deficiency of iron, internal secretion of thyroid, and vitamin C. The second arises with deficiency of the hæmopoietic principle.

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pathognomonic, and a leucoblastic reaction of a non-specific kind

It is surprising that the latter change should occur, because at this stage there is leucopenia with relative lymphocytosis in the blood. This may be due to some inhibition of emigration and also of proper maturation of the granular leucocytes. Certainly it is common to be able to detect abnormalities of both nuclei and cytoplasm in the neutrophils, especially in the metamyelocyte stage (p. 6).

Fallon regards the changes in the metamyelocytes as diagnostic of pernicious anaemia, and Dameshek and Valentine say that the percentage of these cells increases with the severity of the disease. The nature of the changes is uncertain, but on the available evidence we would agree with Jones that the abnormal cells are the result of abnormal development of the myelocytes associated with the deficiency of haemopoietin. Tempka and Braun, who originally drew attention to the altered metamyelocytes, regarded the changes in them as degenerative, and Nordensen concurs with this opinion. The metamyelocytes are increased in size up to 30μ and show large U shaped nuclei with a coarse chromatin network. Cytoplasmic granules are present but only stain well when a pH 6.8 buffer is used for diluting the neat stain (Foy and Kondi).

Further, there is a considerable excess of haemocytoblasts, which, of course, adds to the appearance of leucoblastic activity. In some long-standing, untreated, cases, there may be some depression of granulopoiesis.

There is much difference of opinion as regards the megakaryocytes in this malady, and at the present time it is best to assume that they may or may not show changes. The presence of many naked megakaryocytic nuclei has been commented on (Tempka and Braun), and Jones (1936) found some intensely basophil megakaryocytes with no granules in the cytoplasm and marked pleomorphism of the nucleus. Other workers have merely noted a diminution in the number of megakaryocytes, while Nordensen holds that they are normal in appearance and number.

Japa claims that the essential abnormality in pernicious anaemia is a slowing down of the rate of mitosis with increase in the length of the resting phase and consequent increase in size of the affected cells, all three cell types, erythroblastic, leukoblastic and megakaryoblastic are affected. This change is said to be due to an alteration in nuclear metabolism.

The megaloblastosis is, naturally, the most significant change,

and it is by progressive increase that we can recognise deterioration by diminution, that we see that improvement is occurring and by disappearance that complete relief is established. The cells are easily distinguished from normoblasts, but too much attention should not be paid to their size, which is very variable. The nuclear characters (p. 9) are the differential feature. These cells are found lying in groups, often surrounded by a clear area, devoid of cells, and they seem to develop more or less independently of all other marrow elements. Promegaloblasts are also present, and their number is some guide to prognosis. It is interesting to find that the number of these cells is closely related to that of the hæmocyto blasts, as the one decreases, so do the others. And it is reasonable to assume that they are closely related, and indeed, probably of the same series.

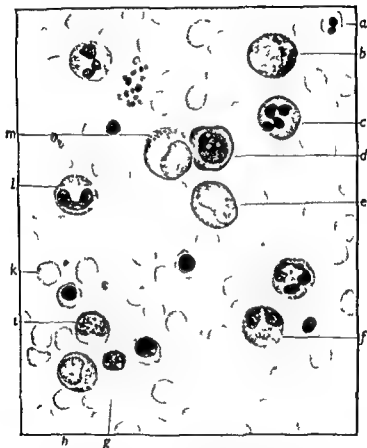
In an untreated case of moderate or great severity, the striking feature of the marrow film is that there is a general immaturity, and the marrow appears to be both hyperplastic and metaplastic, the latter appearance being due to the presence of so many hæmocyto blasts. Indeed, so many of the marrow cells are immature, that, with the ordinary hæmatological stains, the films have a strikingly blue colour, which, by itself, is very suggestive of pernicious anæmia. In myeloblastic leukaemia, the films are far less blue, because the myeloblast has a cytoplasm somewhat less basophilic than the hæmocyto blast and the promegaloblast.

Of course, the other signs of pernicious anæmia to which we attach so much importance in the blood, can also be detected in the marrow. megalocytosis, anisocytosis, Jolly bodies, hyperchromia and polychromasia are always seen. If the marrow fluid is stained with cresyl blue (see Appendix) before the films are made, there is a distinct though moderate increase in the number of reticulocytes.

Mitotic figures are often numerous, and so far as it is possible to be sure, they occur mainly, or perhaps entirely, in the megaloblasts. But the number of atypical mitoses is always great. Some are pyknotic, some multipolar, and others even more grossly irregular. This is pathognomonic of pernicious anæmia, and is never seen in the normoblastic mitoses that may be numerous in post hæmorrhagic anæmia during the phase of regeneration.

In the phases of remission, induced by liver treatment, a series of significant changes can be detected. These start about twenty four hours after intramuscular injection of a potent liver extract, and the first change to be detected is increase in the number of reticulocytes. After 4 c cm. of Neo-Hepatex, we have seen the reticulocytes

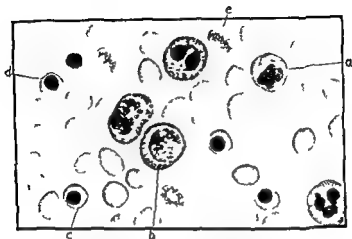
PLATE I



NORMAL MARROW

- | | |
|--------------------------|-----------------------------|
| (a) Normoblast | (g) Basophilic normoblasts |
| (b) Basophilic myelocyte | (h) Pro-erythroblast |
| (c) Polymorph | (i) Early normoblast |
| (d) Myeloblast | (k) Polychromatic corpuscle |
| (e) Myelocyte | (l) Metamyelocyte |
| (f) Eosinophile | (n) Premyelocyte |

PLATE 10



A PERNICIOUS ANÆMIA (LATE STAGE IN TREATMENT)

- | | |
|----------------------|------------------------------|
| (a) Late megaloblast | () Normoblast |
| (b) Myelocyte | (d) Polychromat c normoblast |
| (c) Reticulocyte | |



B IDIOPATHIC HYPOCHROMIC ANÆMIA

Note — Numerous basophilic normoblasts and hypochromic microcytes

rise from 8 per cent of the total non-nucleated elements to 39 per cent twenty seven hours after the injection. Within two days there are signs of a change in type of the erythropoiesis: the intense megaloblastosis is beginning to die down and to be replaced by normoblastosis. The percentage of the latter cells may rise from twenty-two to thirty-seven in fifty hours. Even four to five days after treatment has commenced, the marrow picture is still characteristic of pernicious anæmia. About half of the normoblasts are large and have an intensely basophilic cytoplasm: these are of the early and basophil type of normoblast. The remaining half are composed of equal numbers of polychromatic and eosinophil normoblasts of normal size. Mitosis is still intense, but is now confined almost entirely to the large basophil early normoblasts. Promegaloblasts and basophil megaloblasts are still present in moderate numbers and are easily recognised by their nuclear characters. Very few of them show mitosis. Polychromatic and eosinophil megaloblasts are conspicuous only by their absence. A few megakaryocytes can now be found in the thick end of the film. The activity of the myeloid tissue does not show much change, but in some cases there may be a marked increase in the numbers of eosinophile myelocytes and polymorphs. This may be reflected in the peripheral blood picture.

After seven to ten days the marrow picture becomes normal. The changes in the myelogram, with both anahæmin and folic acid, are shown in Table VI.

It is not at all clear by what means the change in erythropoiesis comes about, but it is probable that it is due to a stimulus to normal maturation of young normoblasts, rather than to fresh formation of cells. The intense karyokinesis seen in these cells supports this supposition, and the failure of hæmoglobinisation of the megaloblastic cells shows that they are no longer required as oxygen carriers.

In the late stages of treatment and in the stage of remission during continued treatment, the marrow film is rather uninteresting (Plate 10A). The megaloblastosis has disappeared, although a very occasional megaloblast may be found before the anæmia has completely disappeared. The myeloid elements form a normal proportion of the film. Normoblasts are present in normal proportions, but there is a tendency for them to become hæmoglobinised earlier, and for the nucleus to be discarded while the cell is still large. This phenomenon probably accounts for the macrocytosis, which still persists in some treated cases of this disease. We have already seen

TABLE VI

BONE MARROW CHANGES DURING TREATMENT OF PERNICIOUS ANÆMIA

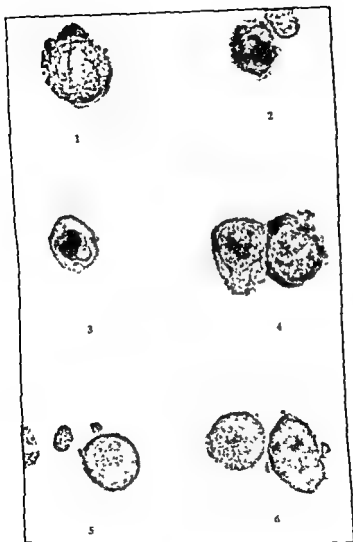
Therapy	Folic Acid 20 mgm. daily for 2 days 20 mgm. daily for 6 days				Anahæmin 1 dose 20 c.c.				
	0	1 day	3 days	26 days	0	6 hours	12 hours	1 day	7 days
Hæmohistioblast	0.6	1.0	0	0.4	0	0	0	0	0
Hæmocytoblast	2.2	1.8	0.4	0.4	0	0.5	0	0.5	0
Myeloblast	2.4	1.2	0.4	0.6	0.5	0.5	0.5	0.5	0.5
Premyelocyte	3.4	0.6	0.8	0.8	0.5	0.5	0.5	0	0.5
Myelocyte	6.6	6.0	5.8	4.0	5.0	8.0	9.5	7.5	14.0
Metamyelocyte									
Polymorphs									
Eosinophil myelocytes									
Eosinophils									
Basophils									
Giant metamyelocytes	12.0	3.4	0	0.2	not recorded			separately	
Pro erythroblasts	1.6	3.0	0	1.0	1.5	0.5	0.5	1.0	0.5
Basophil megaloblast	3.0	8.2	1.2	0	8.0	6.5	7.5	8.0	0.5
Polychrome	3.6	6.0	0	0	12.0	15.5	9.0	10.5	1.0
Eosinophil	3.4	4.4	0	0.4	7.0	25.5	3.5	2.5	0.5
Basophil normoblast	0	3.8	1.6	1.8	1.0	1.0	9.5	25.0	3.5
Polychrome	0	0.6	15.0	5.8	3.0	1.0	4.5	10.0	6.0
Eosinophil	0.2	2.2	5.8	1.6	21.0	9.0	6.5	9.0	17.5
Lymphocytes	28.6	22.4	20.4	25.6	8.0	10.0	6.5	4.5	10.5
Plasma cell	0.4	0.2	0	0	0	0.5	0.5	—	0.5
Monocytes	0	0	2.2	0.2	0	0	0	0	0
White red ratio	1.42	0.53	0.7	0.98	0.69	0.80	1.15	0.42	1.2
Mitosis red cell	0.2	2.4	0.6	0	0.1	0.3	0.4	1.5	0.2
white cell	?	?	?	?	0	0.2	0.2	0.1	0.1

The figures for folic acid treatment have been reported by Harrison and White
The anahæmin figures are our own

that this mechanism may account for some of the macrocytic hæmolytic anæmias, and it may be that there is still some further factor concerned with normoblastic development with which we are not yet acquainted. Reticulocytes in fully treated cases number about 3 per cent in bone marrow films.

The practical purpose of sternal puncture in pernicious anæmia is not usually that of early diagnosis, however, in cases which have become hypoplastic, and have an atypical blood picture, marrow puncture is diagnostic. As has been said, in ordinary cases, blood changes precede detectable alterations in the marrow, but signs of relapse occur in the marrow before they can be appreciated in the blood, they naturally take the form of megaloblastic increase at the

PLATE II



MEGALOBLASTS IN PERNICIOUS ANEMIA

- | | |
|-------------------------------------|-------------------------|
| 1 Proerythroblast and myelocyte | 4 Abnormal megaloblasts |
| 2 Basophilic megaloblast | 5 " " |
| 3 Hemoglobinization of megaloblasts | 6 " " |

expense of the normoblasts. Nevertheless, the most important purpose of marrow examination in this malady is the understanding of the morphological changes that characterise the disease, no other method has cast so much light into the dark places that surround the erythropoietic process as a whole.

There are three other diseases in which an anæmia indistinguishable from pernicious anæmia may occur. These are intestinal infestation with *diphyllobothrium latum*, tropical nutritional anæmia, due to deficiencies in the diet, and the macrocytic anæmia of pregnancy (see p. 54).

The megalocytic anæmias associated with disease of the liver are of the same type as pernicious anæmia, but are rarely severe and will react to liver therapy. If cirrhosis of the liver gives rise to much gastric hæmorrhage the marrow picture will be complicated by a normoblastosis and leucoblastosis as in the hæmorrhagic anæmias. Anæmia associated with lesions of the alimentary canal is rarely due solely to lack of hæmopoietic principle.

The conditions have been classified by Wilson as follows—

- (1) *Deficient intake of extrinsic factor*—tropical and nutritional macrocytic anæmia including the macrocytic anæmia of pellagra.
- (2) *Decreased formation of intrinsic factor*—gastric and intestinal resections and possibly carcinoma of the stomach.
- (3) *Impaired absorption of hæmopoietic principle*—sprue, celiac disease, worm infestations, Crohn's disease and intestinal anastomoses and ulceration.
- (4) *Impaired storage and metabolism of the principle in the liver*—cirrhosis of the liver and possibly pernicious anæmia of pregnancy.
- (5) *Impaired utilisation of the principle in the liver*—achrestic anæmia.

In all the conditions, anæmia may be due to lack of hæmopoietic principle or iron, or commonly a combination of both. The most common anæmia with alimentary disease is certainly a hypochromic microcytic one associated with failure to absorb iron. The myelogram will be fully discussed later. In the combined deficiency, the marrow megaloblastosis is present with the changes due to lack of iron. In these cases marrow puncture may give indications of great therapeutic value in showing that both iron and liver are necessary for adequate treatment. Quite often the peripheral blood shows only the macrocytosis which is treated with liver, only when the hæmoglobin becomes constant at a relatively low figure with a low colour index does the need for iron become apparent. Both thera-

peutic agents could have been given at once if the need for them had been recognised at first (see *Dimorphic Anæmia*, p. 53)

Anæmia in Gastric Carcinoma Currie states that the marrow condition may be megaloblastic, as in pernicious anæmia, normoblastic, as in any ordinary hypochromic anæmia, and erythroblastic

The last named has not been clearly described before. It appears that the marrow is very cellular and active, with many mitoses. The predominant cells are macroblasts, *i.e.*, large, basophilic normoblasts. They may be as large as megaloblasts, but have a definitely 'cart-wheel' arrangement of the nuclear chromatin. The anæmia in patients with this type of marrow is more severe than in those with a megaloblastic reaction.

Currie is of the opinion that the type of marrow reaction is not related to the severity of the gastric lesion, and he also says that there is no obvious correlation between the apparent degree of deficiency of specific hæmopoietic substance and the type of reaction of the erythron. 'Deficiency of specific hæmopoietic substance *per se* does not appear to result in a megaloblastic marrow reaction and a megalocytic anæmia. In 59 cases examined by Monasterio the anæmia was of the normochromic normocytic type with many basophil normoblasts in the marrow.

Achrestic Anæmia In this malady the marrow changes are similar to those seen in pernicious anæmia and the mixed megaloblastic and normoblastic picture seen in partly treated pernicious anæmia is often encountered. Israels and Wilkinson find these observations to be in keeping with the view that in achrestic anæmia there is some inhibition of the action of hæmopoietin on erythropoiesis.

Macrocytic Anæmia in other Conditions A variety of single cases have appeared in the literature in which the primary disease has been unexpectedly associated with a macrocytic anæmia and a megaloblastic bone marrow. No reason is apparent for such associations, but it should be remembered that they may occur. Those most recently recorded are generalised tuberculosis and macrocytic anæmia (Merwe), increased erythrocyte fragility associated with a macrocytic hæmolytic anæmia (Duke and Young), and an acute erythroblastic anæmia of childhood (McLean). Foy and Kondi have reported an extremely unusual case where a megaloblastic marrow was associated with a severe microcytic anæmia of the peripheral blood. The patient was a pregnant Bechuanaland negress suffering

from amœbic dysentery and made a complete recovery after a course of emetine injections

Davis has reported three cases of megaloblastic anæmia in children aged respectively twelve, thirteen and fifteen years. The blood and marrow pictures were characteristic of pernicious anæmia. Two of the cases proved refractory to parenteral liver injections, but responded to proteolysed liver administered by mouth, and Davis suggests that these cases were due to failure of intestinal assimilation and not true examples of pernicious anæmia.

Deficiency of Iron This results chiefly in the malady known as idiopathic hypochromic anæmia (Plate 10B) so clearly depicted by Witts, the nutritional anæmias associated with infancy, the anæmia associated with alimentary disease, and some anæmias of pregnancy. Where the anæmia is solely due to deficiency of iron, the blood shows no spectacular changes and there are never very many normoblasts. The marrow, however, is characterised by a great preponderance of normoblasts, many of which are large, immature, and strikingly basophilic. The whole picture is one of very active erythropoiesis, and mitotic figures are numerous; these are unlike those seen in the megaloblasts of pernicious anæmia, inasmuch as they are all typical abnormal karyokinetic figures are never seen.

Neutrophiles	
Myelocytes	17-22 per cent
Metamyelocytes	5-8 "
Polymorphs	8-12 " "
Eosinophiles	
Myelocytes	2-4 " "
Polymorphs	2-4 " "
Premyelocytes	1-3 " "
Myeloblasts	0-2 " "
Pro erythroblasts	8-10 " "
Normoblasts (basophilic)	20-30 " "
Normoblasts (eosinophilic)	20-30 " "
Megakaryocytes	scanty
Reticulocytes	up to about 6 per cent of the non nucleated elements

The activity of the myeloid tissue is somewhat reduced, with a consequent reduction in the number of myelocytes. This is marked distinction to the microcytic hypochromic anæmia produced by

hæmorrhage. The eosinophiles, however, remain normal in numbers or may show a slight increase.

The myelogram has the characters shown above, which, of course, vary considerably as the result of treatment and as so many patients have been given some iron before they are properly investigated, characteristic pictures are uncommon.

During, and even after, successful treatment with iron, the eosinophiles in the marrow increase considerably, although there may be no noteworthy excess in the blood. In pernicious anæmia, as we have seen, eosinophilia may develop in the marrow and in the blood, but this is usually a sign of overdosage with liver (which is, of course, quite innocuous) but it is interesting to note that eosinophilia is a condition that develops during recovery from several kinds of anæmia.

As in pernicious anæmia, one of the most striking features of the marrow during the phase of recovery is the increase in the number of reticulocytes. These may increase to form 20-30 per cent of the total non-nucleated elements. There is, however, one difference between the two diseases. In pernicious anæmia the reticulocyte response is high if the red cell count is low, and low if the red cell count is high, in hypochromic microcytic anæmia the reticulocyte response depends more on the hæmoglobin level. If this is moderately high, the response is poor, and *vice versa*. In both maladies, the reticulocyte response to therapy is extremely rapid.

Other changes in the marrow during the period of recovery are a gradual restoration of the normal number of neutrophiles and a steady drop in the number of normoblasts. The percentage of basophil normoblasts drops more rapidly, till, when the anæmia is abolished, they and the eosinophil normoblast have reached a normal numerical relationship.

The iron deficiency anæmias of pregnancy are similar to idiopathic hypochromic anæmia in blood and marrow. The nutritional anæmias of infancy show the same changes in the peripheral blood, but here, of course, sternal puncture is not possible.

Deficiency of Vitamin C. Anæmia is not a constant finding in scurvy, but is commoner in adults than in children. The anæmia is normochromic or hypochromic and is cured by the administration of vitamin C, whereas iron has no effect on it. Ascorbic acid is a reducing agent and it is probably because of this that its absence, as in scurvy, leads to anæmia. Normally it plays its part in the gut by reducing ferric compounds to the ferrous state, thus making

them suitable for absorption. In the cases examined by Israëls the sternal marrow showed evidence of diminished erythropoiesis, i.e., the total number of normoblasts was decreased, but their development was normal. During treatment with vitamin C, reticulocytosis develops.

If there has been much hæmorrhage into the tissues with the disease, the peripheral blood may show a microcytic anæmia. The marrow, however, is unable to respond in the usual way, and the myelogram of hæmorrhagic anæmia does not develop until vitamin C has been given. A few cases of macrocytic anæmia with scurvy have been reported, but the sternal marrow findings are not recorded.

Deficiency of Thyroxin The exact relationship of thyroxin to the hæmopoietic tissues is not yet worked out, but it is probable that it acts as a general stimulant rather than on any particular hæmopoietic tissue.

In myxædema, if uncomplicated by deficiency of iron and of hæmopoietic principle, the bone-marrow shows simple hypoplasia. In thyrotoxicosis there is a general hyperplasia of the bone marrow which may affect the myeloid elements to a greater degree than the erythroblastic tissue.

Banti's Syndrome In the early stages of the malady, while the anæmia and leucopenia are not severe, the marrow films show a definite myeloid hyperplasia with a relatively normal ratio of myelocytes, metamyelocytes and neutrophils while no abnormality can be detected in the erythroblastic activity. Later the marrow shows a "maturation arrest" of the hyperplastic myeloid tissue with an increase in the percentage of myelocytes while the neutropenia of the peripheral blood is becoming more severe. At this stage thrombocytopenia may be well marked but the marrow appears to be well stocked with megakaryocytes. In the later stages of the condition with severe macrocytic anæmia and cirrhosis of the liver the myeloid hyperplasia persists, but there is a marked increase in the number of immature normoblasts. In Felty's syndrome (enlargement of spleen and glands, anæmia and chronic arthritis) the marrow changes are similar, but some authors (Limarzi *et al*, Fleischacker and Lachnit) have reported an increase in the number of hæmohistioblasts and plasma cells.

Dimorphic Anæmia In this malady there is a deficiency of hæmopoietin and iron, and it may occur as a complication of a tary disease. In those idiopathic cases which we have peripheral blood picture has been that of pernicious

the administration of liver extracts has been followed by an improvement in the anæmia with a more or less rapid lowering of the colour index to well below normal. Further treatment with liver has no effect and the blood picture is only restored to normal after large quantities of iron have been given. The appearances of the sternal marrow vary considerably. Trowell has examined 174 cases of combined deficiency and says that the predominating type of erythropoiesis is normoblastic although megaloblasts and the large basophil normoblasts of iron deficiency can also be found. In our experience the picture resembles closely that of a partly treated pernicious anæmia with an active normoblastosis and some megaloblastosis, the number of reticulocytes present, however, in the untreated disease is very small. The condition can be distinguished from achrestic anæmia (the marrow changes of which are very similar) by the absence of free acid in the gastric juice. Megalocytic anæmias associated with dietary deficiencies are often dimorphic in type.

Anæmia in Pregnancy This may be normocytic, microcytic or macrocytic. In normal pregnancy, hydræmia occurs and this is accompanied by a degree of normochromic normocytic anæmia of the peripheral blood and myeloid and megakaryocyte hyperplasia of the bone-marrow. That these changes do accompany normal pregnancy is perhaps not generally recognised, and there is a need for the establishment of a set of normal standards during pregnancy.

The true anæmias of pregnancy are mostly due to partial failure of production of hæmopoietic principle owing to temporary derangement of gastric function and the necessity of supplying the principle to the fœtus.

Two types of macrocytic anæmia have been described by Markoff the first is a true pernicious anæmia of pregnancy and the second a macrocytic anæmia in which the marrow show both normoblastosis and megaloblastosis.

Wolff and Lunarzi claim that the type of anæmia in pregnancy should only be diagnosed from marrow examination because the changes in the peripheral blood may be misleading. Apart from a typical iron deficiency anæmia, they have described a macrocytic anæmia with a normal or pro normoblastic marrow and a megaloblastic marrow with macrocytic, normocytic or microcytic and hypochromic anæmia, in the former type recovery follows delivery, and in the latter a good response can be obtained with liver therapy and the conditions disappears spontaneously in the puerperium.

In our experience, all these forms have occurred, but a small proportion of them, including some of those which present a picture of iron deficiency do not clear up after delivery and despite numerous types of therapy have continued to show a moderate degree of anaemia with persistence of the changes in the bone-marrow

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macroblasts or even pro-erythroblasts: There is also some leucopoiesis in the liver, but this is not intimately mixed with the immature red cells

The sternal bone-marrow may appear normal during the greater part of the disease, only becoming involved in the terminal stage. It is important to remember this, for it shows very clearly that it is never safe to assume that, because the marrow is normal, we are not dealing with a disease of the hæmopoietic tissues. It has long been known that the blood may still appear healthy at a time when the formative organs are already abnormal, but it is less widely recognised that the bone-marrow may appear healthy at a time when the blood already shows signs of disorder.

What little evidence is available seems to suggest that erythro-leukæmia is a special mode of reaction to some unknown noxious

Neutrophiles	
Myelocytes	10-18 per cent
Metamyelocytes	5-7 " "
Polymorphs	35-42 " "
Eosinophiles	2-4 " "
Lymphocytes	1-2 " "
Monocytes	8-12 " "
Premyelocytes	0-1 " "
Hæmocyto blasts	1-3 " "
Normoblasts	12-18 " "
Megaloblasts	1-3 " "

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CHAPTER VI

INFECTIVE DISEASES

LEUCOCYTE counts have been of considerable value in medicine and surgery as well as in more purely academic studies of immunity. The blood changes in infective diseases are relatively well known, but the marrow findings have not yet been investigated in much detail.

The types of changes that may occur can be classified as follows —

- (1) Normal marrow with no alteration in the percentage composition
- (2) Hyperplastic marrow with predominance of mature and almost mature neutrophils
- (3) Hyperplastic marrow with many immature neutrophils, mainly myelocytes
- (4) Extremely immature marrow in which premyelocytes are numerous or even prominent
- (5) Myeloblastic marrow

An alternative classification suggested by Barta was —

- (1) Moderate reaction with plentiful cells
- (2) Intense reaction with immature cells including many myelocytes
- (3) Extreme reaction with many premyelocytes or even myeloblasts, and
- (4) Failure of reaction with decrease of granular leucocytes

No rigid classification is possible because there is a continuous series of gradations between the different types of marrow. All that can profitably be done is to call attention to the outstanding characters of the myelogram.

As in the blood so in the marrow, the picture is not pathognomonic of a particular infection. The myelogram, like the hæmogram, gives an indication of the intensity of reaction but not of the underlying cause. In a few infections it is alleged that there are pathognomonic changes, for example in both lobar and bronchopneumonia there is a greater degree of variation in the size of the granulocytes than in health, but this anisocytosis is not visible in the blood and

Normoblasts	25-25 per cent
Megaloblasts	1-3 " "
Megakaryocytes	scanty, but always present

Recently several authors have doubted whether there is, in fact, any true monocytic reaction in the bone-marrow. There is no doubt that marrow smears in this disease often shows a marked increase in monocytoïd cells, but it is possible that this may be the result of admixture with circulating blood and not a specific response of the marrow cells. Perusal of the literature of bone-marrow biopsy in glandular fever indicates that about half of the reports show an increase in monocytic cells and half do not. Limarzi, Paul and Poucher give a good review of this subject and analyse twenty five cases of their own. Their conclusion is that the marrow does show a myeloid hyperplasia and immaturity but is not involved in the production of the monocytoïd cells. The average of the findings in the twenty-five cases is given as follows —

Myeloblasts	0.33 per cent	Range = 0 - 2.25
Premyelocytes	0.82 " "	" 0 - 3.0
Myelocytes	20.0 " "	" 4.5 - 48.0
Metamyelocytes	48.0 " "	" 24.0 - 68.3
Polymorphs	5.55 " "	" 1.5 - 19.5
Eosinophiles	0.78 " "	" 0 - 2.25
Basophils	0.06 " "	" 0 - 1.5
Proerythroblasts	1.70 " "	" 0.75 - 7.5
Basophil Normoblasts	2.08 " "	" 0.5 - 4.25
Polychrome "	21.8 " "	" 19.0 - 24.0
Eosinophil "	0.5 " "	" 0 - 1.75
Monocytoïd .	2.5 " "	" 1.0 - 6.0

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HYPOPLASIA AND APLASIA OF THE BONE-MARROW

APLASIA of the bone-marrow may arise from a variety of causes, ranging from exhaustion, to poisoning or to lack of specific nutritional factors. A heterogeneous group of diseases is involved including anaemia, agranulocytosis, and purpura, or a combination of all three, according to which part of the haemopoietic tissue is involved. Sternal puncture has been of great use in elucidating the pathology of these conditions and has shown that the peripheral blood may give a very inaccurate reflection of the conditions in the haemopoietic bone marrow. This tissue may be truly aplastic with failure of division of the primitive cells (primary aplasia), or there may be normal division of the precursor cells resulting in a very cellular marrow, which, owing to lack of some maturation factor, is unable to furnish finished elements to the blood stream (maturation type).

Whitby and Britton have made a useful classification of these diseases based on the marrow findings in the separate or combined affections of the erythroblastic, leucoblastic and thromboblastic tissues —

1 Aplasia of the erythroblastic tissue

- (a) Primary—pure red cell anaemia. The authors have found only five cases in the literature.
- (b) Maturation defect—dyshæmopoietic anaemias may become aplastic if the maturation factor is never supplied.

2 Aplasia of the leucoblastic tissue

- (a) Primary—agranulocytic angina.
- (b) Maturation defect—agranulocytic angina with marked myeloid reaction in the marrow. These cases respond more readily to pentnucleotide therapy, which possibly supplies some maturation factor.

3 Aplasia of the thromboblastic tissue

- (a) Primary—essential thrombocytopenic purpura. An acute form in which there is no rise of platelets after splenectomy.
- (b) Maturation defect—essential thrombocytopenia. Splenectomy is probably beneficial in this type by removing a factor which inhibits platelet maturation.

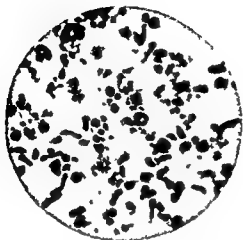
the predominant cells in most cases of this type of aplastic anæmia, and there is also a considerable excess of monocytoid elements, many of which are obviously phagocytic. But it is always difficult, and sometimes impossible, to classify accurately lymphocytes, monocytes and pathologically altered myelocytes. Davidson calls these unclassifiable elements "Q" cells. If myeloblasts are present they are always abnormal, commonly they have indented nuclei, which may even be lobulated (Reider type).

In one case of idiopathic aplastic anæmia recently seen, the marrow showed some hypoplasia of all the nucleated elements (which were otherwise normal) and gross degenerative changes in the erythrocytes which assumed bizarre forms in the films and even appeared fragmented. In the peripheral blood, however, the erythrocytes appeared normal except for a slight macrocytosis (Fig. III).

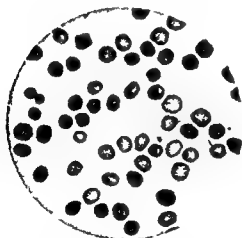
In aplastic anæmia with a hypercellular marrow the clinical picture is indistinguishable from that with a hypoplastic marrow. We have during the last two years had under observation a girl of sixteen years whose peripheral blood shows a progressive normocytic anæmia with no change in the white cells. The whole variety of hæmatinic drugs has no effect on her condition and every three or four months she is transfused with 2 or 3 pints of blood. This raises her hæmoglobin to between 60 and 80 per cent. Haldane, which level she will maintain and sometimes improve slightly for about a month. It then gradually falls to between 30 and 40 per cent, at which level the transfusion is repeated. Sternal puncture has always shown a hypercellular marrow with a normal differential count except of a slight persistent increase in the megaloblastic red cell series. A characteristic count from this case is as follows:—

Hæmocytoblasts	0.5 per cent.
Myeloblasts	1.0 " "
Premyelocytes	1.0 " "
Myelocytes	13.0 " "
Metamyelocytes	22.0 " "
Neutrophils	12.0 " "
Eosinophils	2.0 " "
Basophils	0.5 " "
Pro-erythroblasts	0.5 " "
Basophil megaloblasts	0.5 " "

FIG III



A



B

APLASTIC ANEMIA

- A Fragmentation of red cells in marrow
B Normal cells in peripheral blood

Polychromatic megaloblasts	15 per cent
Eosinophil megaloblasts	15 " "
Basophil normoblasts	55 " "
Polychromatic normoblasts	60 " "
Eosinophil normoblasts	135 " "
Lymphocytes	150 " "
Plasma cells	10 " "
Unidentified cells	30 " "

Any of the agents that can cause aplastic anaemia, e.g., benzol, arsenobenzene, thorium, the more modern sulphur compounds, X-rays, etc., may in smaller concentrations (or after less prolonged exposure) cause a less severe hypoplastic anaemia. It is unfortunate that, although such stages are, at first at least, less grave, they tend to deteriorate, and recovery is very uncommon. All these substances tend to attack the leucoblastic tissues first and the erythroblastic tissues later. X-rays behave in the same way. With sulphapyridine the effect on the leucoblastic tissue may be so severe that death ensues before any anaemia makes itself apparent, and these cases are often classed as agranulocytosis. Benzol commonly attacks the leucoblastic tissue first, but cases have been recorded in which the platelets were first affected. Even when there is a marked leucopenia, due to benzol, there may be some excess of eosinophiles both in the blood and in the marrow. These changes also occur with drugs containing the benzol ring, e.g., arsphenamine. Six cases of blood dyscrasia with characteristic marrow changes after the exhibition of arsphenamine have been reported by Ferguson.

It is important to realise that the myelogram can give us very early information in cases of suspected poisoning of the bone marrow with these compounds. The number of people working with benzol has increased enormously during the years of the war, and we have had the opportunity of examining large numbers of these. In some workers, showing the typical clinical syndrome of benzol poisoning, the hæmatological findings have proved negative. Sternal puncture, however, may show definite alteration in the hæmopoietic tissue. The myelogram quoted below was found in a female benzol worker, aged thirty-three, who had complained of dizziness, lassitude and menorrhagia for some four weeks. Her blood count showed a moderate microcytic hypochromic anaemia (Hb 68 per cent, R B C 4,000,000, C I 0.85). The white blood count was 5,500 per cubic millimetre and the differential —polymorphs 61 per cent, lympho-

cytes 32 per cent, monocytes 4 per cent, eosinophiles 3 per cent
The myelogram was as follows —

Neutrophiles	
Myelocytes	14 per cent
Metamyelocytes	10 " "
Polymorphs	23 " "
Eosinophiles	
Myelocytes	15 , ,
Polymorphs	10 " "
Lymphocytes	265 , ,
Monocytes	45 "
Myeloblasts	25 " "
Normoblasts	17 " ,
Megakaryocytes	very scanty
Reticulocytes	15 per cent of the non nucleated elements

Here we see at once some depression in the myeloid tissue. Normoblasts are present in normal numbers, but not in such large quantities as one would expect if the anaemia were due to iron deficiency. The low reticulocyte count is striking. The number of lymphocytes is raised, and this point brings one to an interesting speculation. We know that it is not possible at the moment to determine the activity of the bone marrow by doing cell counts on the sternal marrow fluid, but we may assume that if there is no absolute lymphocytosis or lymphopenia in the peripheral blood, an increase in the percentage of lymphocytes in the bone marrow is only a relative increase, and that there is no alteration in their absolute number. Then, an increase in the percentage of lymphocytes can be used as an index of the decrease in the amount of the other elements of the marrow. In the case quoted above, the absolute number of lymphocytes in the peripheral blood is within normal limits (1,820), in the marrow, however, the lymphocytes are increased one and a half to two times. It would therefore seem reasonable to assume that the myeloid and erythroblastic elements are reduced to the same extent, as there is no valid reason to account for an absolute increase of lymphocytes in the bone marrow. Whether this inference is admissible or not, the other observation that this marrow showed a general depression compatible with benzol poisoning, before any typical changes in the peripheral blood were noted, is of diagnostic value.

When anaemia due to benzol poisoning is fully established well-

marked changes are always present in the bone marrow. Mallory, Gall and Brickley have investigated a series of cases and found that between 30 and 40 per cent showed severe hypoplasia of the sternal marrow (no cases of complete aplasia were seen), about 40 per cent showed normal cellularity and about 20 per cent showed a hyperplastic condition. In the hypoplastic type the myelogram was substantially the same as that already quoted (p. 68), and one case showed a high proportion of plasma cells, some with multiple nuclei. In the normocellular group there was an increase in the proportion of granulocytes and megakaryocytes with what the authors call numerous "clumps of hæmocyto blasts". In the hyperplastic type proliferative activity was sometimes extreme with not infrequent multipolar mitoses, the hæmocyto blasts and megakaryocytes were also increased in number, the latter showing abnormal forms. Erf and Rhoads have reported similar findings and refer to the increased number of "primitive" cells from 4 to 34 per cent. It must be remembered that leukaemia may develop after benzol poisoning and the marrow then shows the changes ordinarily seen in leukaemia. Mallory *et al* and Erf and Rhoads quote typical cases in their papers.

One further case may be quoted where changes in the sternal marrow were detected before they appeared in the blood. A male,

polymorphonuclears were seen in the film. Sternal puncture was

blasts numbered only 12 per cent. The lymphocytes were 25 per cent. The polymorphs were very degenerate, and there were only 10 per cent of myelocytes. Here we had a primary aplasia of both the leucoblastic and erythroblastic tissue, the latter not yet being apparent in the blood. Two days later the hæmoglobin and the red cell counts began to fall and the patient died in spite of repeated transfusions with fresh blood.

One more point remains to be mentioned in connection with this group of marrow poisons. It would seem that only susceptible persons are affected. Most cases of fatal poisoning with benzol have

large numbers (perhaps normal numbers) of the less mature forms of granulocytes, but practically no polymorphonuclears, and the peripheral blood shows the typical picture of extreme leucopenia, with relative lymphocytosis. Of the granulocyte precursors, Jaffe has found that the myeloblasts show no morphological abnormality, but the myelocytes show degenerative changes in the nucleus which eventually lead to the death of the cell (Plate 12A). The nuclear changes are preceded by some splitting and degeneration of the cytoplasmic granules. Jaffe emphasises these changes as the specific pathological features of the malady, and it would seem that they depend upon the absence of some specific factor that is normally required for the proper differentiation of the myelocytes. If this factor could be found, the proper cure could be found. The available

substances as sodium pentose nucleotide is often followed by cure. But in the aplastic form, there is little evidence that treatment on these lines is of any value at all. The myelogram in the "maturation" type of agranulocytosis is of the following type —

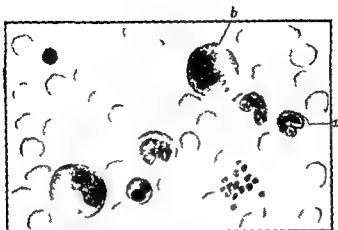
Neutrophiles	
Myelocytes	30-35 per cent
Polymorphs	1-5 " "
Eosinophiles	
Myelocytes	1-2 "
Premyelocytes	1-2 " "
Hæmocytoblasts (including myeloblasts)	1-2 " "
Lymphocytes	20-35 " "
Normoblasts	15-20 " "
Megakaryocytes	scanty

The myelocytes show degenerative changes in the nucleus and specific cytoplasmic granules.

Whether the aplastic type of agranulocytosis is an advanced stage of the "maturation" type is still unknown, but it is obvious that, in spite of this large gap in our knowledge, sternal puncture has of the utmost prognostic value. By it we can determine whether there is any hope that treatment will do good, or whether, with the means available, death is inevitable.

Essential Thrombocytopenia In this malady, as with agranulocytosis, there would seem to be a primary aplastic form as well as a "maturation" type resulting from failure of the mega-

PLATE 12



A ACRA LOCTOS 5 (NAT RAT ON TYPE)

No e Abs n e of pu morphs non granula ty of me am elocys (a)
nu les degen on n myelocys (b)



■ THRO BOCYTOPH A (M TIRY ON TYPE)

No e Abnormal megakaryocyte (a) and giant platelet (b)

karyocytes to develop properly. Clinically the aplastic form is acute illness and splenectomy is attended by a 70-80 per cent mortality. In the chronic recurring type or phase of the malady marrow often shows a maturation defect, and in these cases splenectomy practically always alleviates the condition. This has led to supposition that the spleen in these cases produces an inhibitory factor which acts on the bone-marrow.

In the primary aplastic form of the disease, the significant change is the complete absence of megakaryocytes and the paucity of platelets. Careful search of even the ends of the films fails to reveal fragments of megakaryocytes and such platelets as there are may be deformed. If any anaemia has resulted from extensive purpural bleeding the myelogram will show a normoblastic hyperplasia, this is to be regarded as a reaction to the loss of blood and not part of the malady itself.

In the "maturation" type, megakaryocytes are present in normal numbers and show some alteration in structure. Platelets are deficient, however in number and quality, giant forms are more common than in normal marrow (Plate 12B). The megakaryocytes, although as large as fully differentiated forms present much less nuclear lobulation and contortion. The cytoplasm is often vacuolated and may be completely free from granules, whether this is a sign of degeneration, as some writers have contended, is quite unknown. The one point that suggests the megakaryocytes are, indeed, the seat of degenerative changes, is that the number of platelets, both in the marrow and in the blood, is greatly reduced, and it is reasonable to suppose that this may be due to a lack of functional activity of the parent forms.

Dameshek and Miller have counted the number of megakaryocytes in bone-marrow biopsies in idiopathic thrombocytopenic purpura in normals, and in cases of thrombocytopenic purpura associated with various types of splenomegaly. Their figures are as follows -

- 1 Normals—not more than 300 megakaryocytes per million nucleated red cells, 69 per cent showing platelet production.
- 2 Chronic idiopathic thrombocytopenic purpura—increased number of megakaryocytes with decreased platelet production.
- 3 Splenomegaly of non leukaemic origin (cirrhosis, Gaucher disease, etc.) slightly increased megakaryocytes with normal platelet production.
- 4 Aplastic anaemia, lymphosarcoma, acute leukaemia—g

reduction of megakaryocytes a normal percentage of which show platelet production

Although a hypoplastic or aplastic condition of the bone marrow ■ not involved, other forms of purpura may well be mentioned here. Sternal puncture is valuable because there are so many conditions that can give rise to purpura, and their accurate differentiation is of great therapeutic importance. Thus we may find a myeloblastic overgrowth of the marrow which demonstrates that we are dealing with a leukaemia, or, we may discover a marrow that is entirely devoid of erythroblastic and granulocytic elements, and we realise that the malady is some form of aplastic anaemia.

In the purpuras associated with infection or with the action of poisons, there may be a transient increase in the number of megakaryocytes, but quite soon these elements become scanty. Moeschlin, however, could find no changes in the bone marrow of people who developed thrombocytopenia after the administration of sedormid. The myelogram is of the following type —

Neutrophiles	
Myelocytes	10-15 per cent
Metamyelocytes	4-6 , ,
Polymorphs	18-25
Eosinophile polymorphs	2-4 , ,
Premyelocytes	2-4 , ,
Lymphocytes	1-3 , ,
Monocytes	1-3 , ,
Plasma cells	1-3 , ,
Pro erythroblasts	3-5 "
Normoblasts	30-45 "
Megaloblasts	2-4 , ,
Megakaryocytes	1-4 , ,

There seem to be several conditions in which the condition of the peripheral blood suggests that there is hypoplasia of one or all the components of the myeloid tissue, but in which marrow puncture reveals intense hyperplasia. Thus, excessive thrombolysis in the spleen can produce the picture of thrombocytopenic purpura, although there is increased formation of platelets in the marrow. And Doan and Wright have described a condition of *splenic panhaematopenia* in which every type of marrow cell ■ increased in number, although the blood picture suggests the opposite of this

They adduce evidence that, in this condition, there is indiscriminate destruction of every kind of formed haemic element as it passes through the spleen.

Cases which are clinically indistinguishable from essential thrombocytopenia may occur as "allergic" manifestations, particularly after streptococcal infections. Schwartz has suggested that examination of marrow smears may differentiate this condition from the idiopathic type because in the former there is an increased number of eosinophils. This eosinophilia of the marrow in the infective type is not necessarily reflected in the peripheral blood.

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SOME PROTOZOAL DISEASES

It would seem that with these diseases there is an extensive and promising field of work in connection with sternal puncture, as many of the parasites make their home in the reticulo-endothelial system, and so one would expect to find them frequently in the bone marrow. Also the literature on the subject is scanty. The enlargement and softening of the spleen, which so often accompany these diseases, may make spleen puncture an operation of some consequence because of the possibility of persistent bleeding. Liver puncture, though safe, does not usually give very satisfactory material for examination. Sternal puncture, however, is rapid, safe, and provides material which not only may contain the parasites in large numbers, but at the same time provides an opportunity of explaining the more or less characteristic blood changes.

Kala Azar (Plate 13A) Let it not be supposed that this disease is confined to the tropics, many cases from the margins of the Mediterranean basin and from the Sudan have been recorded, and one is tempted to presume that even more have been overlooked.

The myelogram has remarkable characters, inasmuch as there is lymphocytosis, increase of monocytes, intense reticulo-endothelial hyperplasia, patchy deposition of platelets and, of course, the pathogenic agent, the *Leishmania* itself. The last is found lying inside monocytes, but some lie free and rather closely resemble platelets, with which they may easily be confused. Close examination shows

is probably an artefact due to rupture of the cells during spreading of the film. In the films we have seen, where a group of *Leishmania* is encountered, they are always entangled in a faintly basophilic web, probably representing the remains of the monocyte cytoplasm. Quite often a degenerate nucleus is found in close proximity. Extracellular forms must occur in the passage from cell to cell, but these are rare and probably occur singly. In histological preparations, where the cells remain intact, the parasites are almost invariably intracellular. In films also they may sometimes be seen superimposed on a red cell, and they have been thought to lie within them, as with the malarial parasite. This is an artefact. The organisms may be found in peripheral blood films but always within either the monocytes or the polymorphonuclears.

In man the parasite is in a non-flagellate stage. It is circular, oval, or cigar-shaped in outline and is 3 to 4 microns long and 1 to 3 microns wide. The cytoplasm, faintly basophilic, is contained in a tenuous membrane and contains two characteristic structures, which are essential to its recognition. The one, the nucleus, is larger than the other, and is usually spherical. Its diameter is about one-third to one-half of the shortest diameter of the organism. It usually lies against the membrane and is flattened on this side. The flattening may be extreme so that the nucleus appears as a thick line, and this appearance may be accentuated by the presence of vacuoles within the cytoplasm. With Jenner-Giemsa stain the nucleus appears as a bright red granular mass. The second, the blepharoblast, is a short rod-shaped structure with one end pointing to the nucleus. It may appear as a dot if its long axis is perpendicular to the slide. The blepharoblast stains purple with Jenner Giemsa (Plate 134).

Quite apart from the presence of *Leishmania*, the myelogram is characteristic, and may remain so for many months after suitable treatment has caused the disappearance of these organisms.

Neutrophiles	
Myelocytes	18-24 per cent
Metamyelocytes	0-1 " "
Polymorphs	8-10 " "
Eosinophiles	0-1 " "
Lymphocytes	
Small	18-22 " "
Large	18-22 " "
Monocytes	14-18 " "
Plasma cells	1-3 " "
Hæmohistioblasts	0-1 " "
Hæmocytoblasts	0-1 " "
Normoblasts	3-5 " "
Megaloblasts	1-3 " "

In *Leishmania tropica* infections (oriental sore) the lesions are usually confined to the skin, sometimes appearing in the mouth also. Enlargement of the glands may occur if lymphadenitis is present and the organism has been found in them. Very rarely it has been demonstrated in the peripheral blood, but further investigation may show it to occur more frequently in the bone-marrow.

Malaria (Plate 135) In benign tertian and quartan infections the

asexual life-cycle of the parasite takes place in the peripheral blood. In malignant tertian fever, the cycle takes place in the tissues. It would appear, from sternal puncture films of a case of cerebral malignant tertian malaria, that this schizogony occurs, in part at least, in the bone-marrow. All the changes from the signet-ring stage to the rosette stage could be traced, while peripheral blood films showed only a few signet-ring and gametocyte forms. The number of infected corpuscles in the marrow exceeded by far those in the bloodstream. Plate 13B was prepared from this film.

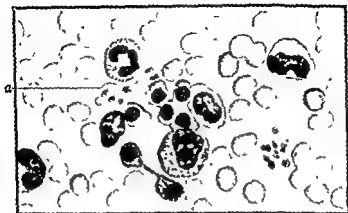
Recently several papers have come from the Middle East showing that sternal puncture may be of great value in the diagnosis of malaria. Aitken examined 95 cases in which serial thick drop preparations made from the peripheral blood at six-hourly intervals had proved negative. In 39 of these cases the parasites were found in the sternal marrow. In a further series of 10 cases which did show the parasites in the peripheral blood, parasite-containing red cells were from 2 to 5 times more numerous in the marrow fluid. Rumball and his co-workers point out that most cases of pyrexial malaria can be diagnosed from the peripheral blood, but that sternal puncture forms a valuable supplementary diagnostic method in those cases where sporulation is scanty and parasites cannot be found in the peripheral blood.

In response to the hæmolysis of red corpuscles by the malarial parasite, the marrow response is essentially normoblastic and monocytic—changes that do not disappear for many months after recovery. Monocytosis, which is considerable in chronic cases, is less marked in acute ones. The following myelogram is of a fairly characteristic type.

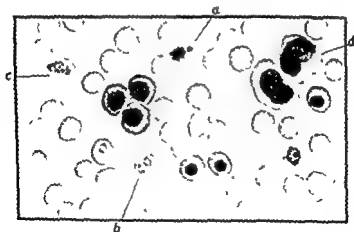
Neutrophiles	
Myelocytes	5-7 per cent
Metamyelocytes	0-2 " "
Polymorphs	9-12 " "
Eosinophiles	0-2 " "
Monocytes	10-14 " "
Premyelocytes	0-2 " "
Hæmocyto blasts	0-1 " "
Normoblasts	40-50 " "
Macronormoblasts	12-8 " "

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A KALA AZAR
(a) *Leishmania* in ruptured monocyte



B MALARIA (MALIGNANT TERTIAN)
(a) Schizonts (c) Young gametocyte
(b) a ring form in a corpuscle (d) Monocytes

THE TECHNIQUE OF STERNAL PUNCTURE

Anatomy. The cartilage of the second rib joins the side of the sternum at the junction of the manubrium and the body. This junction can be felt, and often seen, as a transverse ridge on the front of the chest, as the two bones are slightly thickened at their point of union. The ridge can usually be felt, in the adult male, about 2 inches below the jugular notch. In the female, the manubrium is longer and the ridge consequently lower. Until late adult life the

surface of the manubrium which adjoins the body of the sternum

The centre of ossification in the manubrium appears during the fifth month of foetal life, and at three years of age the bone contains a moderate amount of red marrow. Despite this, sternal puncture is an unsatisfactory procedure if performed much before the age of seven to eight years. The needle enters the manubrium very easily but it is difficult to obtain any marrow at all. Kato, however, prefers to puncture the body of the sternum, which, he states, contains cellular marrow even in infants of one or two years of age. Even so, it is better, in the rare cases in which marrow examination in an infant is necessary, to obtain the specimen by trephining the tibia.

Technique of Sternal Puncture. This is a comparatively simple procedure, but in order to cause as little pain as possible, a few details must be adhered to.

The type of needle to be used is of some importance, because it needs to be thick and strong, so as neither to bend nor break in piercing the bone. Something in the nature of a very short French lumbar puncture needle, with an obturator stylet, is best, and a movable guard on the needle itself is useful to avoid the danger,

suction is well to use a large syringe, e.g., about 20 or 25 c.c.

It is probably not of much importance where the bone is punc-

tured, but as has been said, the thinnest spot lies on the under (caudal) surface of the manubrium—that is in the upper surface of the joint that forms the angle of Luis. And this is the easiest and least painful site for the purpose



STERNAL PUNCTURE NEEDLE
(WITTS TYPE)

The needle resembles a short thick lumbar puncture needle with obturating stylette and movable guard. The guard is so adjusted that there is no possibility of piercing the posterior wall of the sternum. If however it is found that the guard has been placed so near the point that the anterior wall is not pierced it can be moved after the needle is in the tissues.

Now the procedure is as follows: the patient is placed on his back, with a pillow under the shoulders, so as to throw the head backwards. Then novocaine is injected into the site of election, taking care that the deeper tissues, including the sternal periosteum, are infiltrated.

The needle is plunged in just below the angle of Luis, and when the point is felt to reach this it is pushed a little further until it penetrates the small piece of cartilage in the joint. When it seems to be in about the middle of this the butt of the needle is lowered until it is almost touching the chest, and then it is pushed straight upwards until the bone is pierced. The manoeuvre is sometimes accomplished more easily if a rotatory motion is imparted to the needle as in using a bradawl. The sensation obtained when the outer layer of compact bone is penetrated is rather like that of a lumbar puncture when the point of the needle enters the theca. Now the stylette is withdrawn and the needle pushed onwards, thus collecting marrow tissue in the lumen.

Another method in common use is as follows. The guard of the needle is set just above the point of the needle, which is pushed vertically against the anterior plate of the

manubrium. Then, when the resistance of the bone is felt, the guard on the needle is set about 5 mm above the skin surface, the needle is then pushed onwards. If no marrow is obtained the

guard may be raised another 2 mm and the needle again pushed onwards, but the needle must never be pushed so far as to touch the posterior lamella of the sternum. As the thickness of the cancellous bone is between 0.5 and 1.5 cm (Arjeff), there is no danger of penetrating the sternum by this method.

A good deal of information can be obtained, after some experience, by the different sensations obtained as the needle moves onwards. Thus the hard push needed in osteosclerosis is very different from that required by the soft marrow of a leukaemia.

The syringe, either thoroughly dry or rinsed out with normal saline, is now attached to the needle and the plunger pulled out. However well the tissues have been anaesthetised, there is now always an unpleasant sensation, which some patients allege amounts to pain, but it is quite transient. Thick blood like material should enter the barrel of the syringe, but some marrows are too dense for this to occur: even then a sausage of myeloid tissue will usually be found, often mixed with spicules or cancellous bone, lying in the lumen of the needle. Suction is now stopped, and after removing the needle the puncture wound is sealed with collodion.

It is best to spread the whole of the contents of the syringe on a slide.

It is impossible to find any fragments, and then films must be made from the thick fluid. This is often difficult, as all but the most hyperplastic marrows contain globules of fat, which render the preparation of really good films difficult, and, of course, the spicules of cancellous bone make it even more so. For this reason a large number of films should be prepared, and among them good ones will be found. The films should be made as quickly as possible because marrow clots as rapidly as blood, but no attempt should be made to make the films as thin as those of blood, if this is tried, far too many cells will be ruptured in the attempt. It is often possible to make thinner films by mixing some of the marrow juice with a little serum. As the cells in thin films are further apart, this procedure simplifies the performance of the differential count.

Turkel and Bethell have introduced a small trephine with which marrow can be obtained from the sternum with no more trouble than by the usual aspiration method. As material for histological examination can be obtained by this means, it is specially useful.

in cases of myelosclerosis (p 19) In most other cases, aspiration is probably the method of choice

A warning must be given here about the use of post mortem specimens of marrow fluid Normally, gross degenerative changes have taken place in the cells three hours after death and the nuclei of all the cells stain a homogenous purplish blue colour with no visible chromatin pattern, the cytoplasm has often disintegrated completely so that bare nuclei, some surrounded by granules can be seen

The sternum is not the only bone from which marrow can be obtained by puncture Loge has called attention to the ease with which marrow can be obtained from the spinous processes of the third and fourth lumbar vertebræ The technique is much the same as that of sternal puncture, but the guard is set at 1.5 cm then, if no marrow is obtained by suction at this depth, it is safe to push on to a maximum of 2.5 cm

Loge found that the myelograms from sternal and spinous marrow are practically identical, and the same is true of marrow obtained by puncture of the iliac crest Clearly, this knowledge is of great value when serial punctures have to be performed on the same patient

Ordinary films can be stained by whatever method is desired, but as a rule it is, of course, sensible to use the same method as is used for blood, in this way comparison is considerably facilitated On the whole, Jenner's solution is not satisfactory, because the nuclear detail is poorly displayed, and because any azurophilic granules remain unstained Leishman's solution or the excellent Jenner-Giemsa method is very suitable In combined staining May-Grunwald may be used instead of Jenner, and Panchrom stain or Kardos mixture in place of Giemsa Different effects can be obtained by using different combinations, for instance, May Grunwald and Kardos mixture gives extremely brilliant granule staining

Vital Staining (a) Reticulocytes A clean slide is flooded with an alcoholic solution of cresyl blue This film is allowed to dry, and any tendency to roughness on the surface of the dye film is smoothed by gently polishing with a piece of soft paper One small drop of marrow fluid is put on the slide and gently stirred with a glass rod, taking care not to spread the marrow over too large an area so that drying takes place As the marrow fluid mixed with cresyl blue does not clot for some time, it is allowed to stand for

two or three minutes so that maximum staining of the reticulum is obtained. Films are then made from this drop and allowed to dry in air. They can be examined directly for reticulocytes or counter stained for making permanent preparations. Counterstaining however, tends to hide the reticulation in cells which have only a little of the vitally stainable basophilic substance.

(b) Fresh preparations. The most commonly used dyes for this purpose are neutral red chloride and Janus green. The preparations are made in the usual way. Equally good results, however, can be obtained by dark ground illumination of fresh preparations as described below.

Dark Ground Illumination (Fig IV) A good account of this technique was given by Whitby and Hynes. Thoroughly clean and degreased slides and coverslips must be used. A small drop of marrow fluid is placed on a slide and covered with a cover slip, the preparation is immediately sealed with wax and kept warm in the incubator. The slides should be covered so that light is excluded. If good results are to be obtained, the marrow films must be as thin as possible, the smaller the drop of marrow, the thinner the final preparation and, as a rule the drop should be of such a size that when the cover slip has settled, the film does not quite reach its edges. In this way one attains the maximum capillary attraction between the slide and the cover whereas if there is enough marrow fluid to reach the edges of the coverslip, this tends to float up and make a thick preparation.

After half an hour in the incubator, the preparation is examined with dark ground illumination and, if possible some form of warm stage. Under proper conditions the movements of the motile cells are easily seen, as are also the mitochondria and other elements seen in any vitally stained preparations. Dark ground illumination has further advantages in that the cell and nuclear membranes are easily visible and that nuclear structure is also quite clear. The motility of cells is rapidly slowed down by light, so the preparation should only be illuminated for a few minutes at a time, and then allowed to recover in the dark. For observing cell structure the films last quite well for four to six hours without any protection or warming.

The characters of the cells are as follows (see Fig IV)

(1) GRANULOCYTE SERIES (A) *Myeloblast*. A rounded cell 8 to 10 microns in diameter. Thin defined cytoplasmic rim, nuclear membrane equally well defined and somewhat thicker. Nucleus rounded and appears filled with a fine dust, nucleoli appear as

circular outlines where the dust like particles are thicker. Cytoplasm contains mitochondria which appear as faint dots. Cell non motile.

(B) *Premyelocyte* Ten to 20 microns in diameter and rounded, cytoplasmic rim, nuclear membrane, and nucleus as in the myeloblast. The mitochondria are now grouped round the nucleus and appear as thin, wavy rods, sometimes showing Brownian movement. Cell non motile.

(C and D) *Myelocyte* Ten to 20 microns in diameter. The specific granules appear in the cytoplasm of the premyelocyte in increasing numbers first among the mitochondria. Eventually they fill the whole of the cytoplasmic space and may partly obliterate the nucleus which has now no nucleolar structures. The specific granules of each of the neutrophile, eosinophile, and basophile myelocytes are described under the mature polymorphonuclear cell.

(E) *Neutrophile Polymorphonuclear* An active motile cell, 12 to 20 microns in diameter when at rest. The nucleus, now lobed has the cytoplasm filled with the leading end of each of granules.

(F) *Eosinophile Polymorphonuclear* Less actively motile than the neutrophile cell and of about the same size. The granules are readily distinguished as they are larger, appear as rings of light, and may be oval.

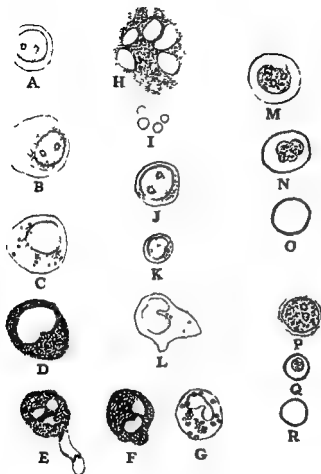
(G) *Basophile Polymorphonuclear* A smaller cell 7 to 10 microns in diameter. The granules are of the same type as the eosinophile granules, but are much fewer in number and may appear even larger.

(2) LYMPHOID SERIES (J) *Lymphoblast* A rounded cell, 8 to 10 microns in diameter. The nucleus is indistinguishable from that of the myeloblast. The two cells however, are easily separated by the appearances of the mitochondria. These in the lymphoblast are larger, less numerous, and definitely rod shaped. Cell non-motile.

(K) *Lymphocyte* Rounded, may occasionally be motile and vary in size from 10 to 15 microns. The nucleus is round or indented and filled with fine dust like granules. The mitochondria are grouped to one side of the nucleus and appear as fine round oval dots. Two three four or five large bright granules are present. "Gall's mahogany granule" shows as a refractile ring of light.

(3) THROMBOBLASTIC SERIES (H) *Megakaryocyte* A large cell up

FIG. IV



DARK GROUND ILLUMINATION OF MARROW CELLS

- | | | |
|------------------|-------------------|----------------------|
| (a) Myeloblast | (g) Basophile | (m) Promegaloblast |
| (b) Promyelocyte | (h) Megakaryocyte | (n) Megakaryocyte |
| (c) Myelocyte | (i) Platelets | (o) Megalocyte |
| (d) Myelocyte | (j) Lymphoblast | (p) Early normoblast |
| (e) Neutrophile | (k) Lymphocyte | (q) Late normoblast |
| (f) Eosinophile | (l) Monocyte | (r) Normocyte |

See p. 74

to 40 microns in diameter. The nucleus usually appears as a group of separate lobes, each of which has a definite nuclear membrane, and is filled with fine dust-like particles. The cytoplasm has no definite rim and is filled with small moderately bright granules.

(1) *Platelets* These appear most as small circles of light, 2 to 5 microns in diameter. The larger number appear to be empty, but some contain five or six small bright granules, which show active Brownian movement, apparently bouncing from side to side of the cell.

(4) **ERYTHROBLASTIC SERIES** (M) *Promegaloblast* A non-motile cell about 10 microns in diameter. The cytoplasmic rim is thick and well defined. This cell shows marked pleomorphism. The cytoplasm appears completely free of granules and other structures although there may be a group of small rounded mitochondria adherent to one side of the nucleus, when this occurs, the nuclear membrane is absent at the point of contact. This membrane is otherwise well defined and thin. The nucleus is filled with fine dust-like particles which are more closely packed and appear much brighter than those of the leucoblastic cells. Also the nuclear particles show some small areas of condensation which appear as brighter granules. The nucleoli, two or three in number, are outlined by a ring of such condensation.

(N) *Megaloblast* Very variable in size and shape. There is a thick cytoplasmic ring and clear cytoplasm without mitochondria. The nucleus may appear lobed and shows areas of localised condensation.

(P) *Early Normoblast* The nuclear structure is the same as in the megaloblast, and the nucleus is of much the same size. The cytoplasmic ring is not well marked, and the cytoplasm is scanty and may contain a few dust-like mitochondria. The cell is easily

(K) *Normocyte* This appears as a bright ring of light with a thick cytoplasmic rim. The megalocyte (O), though larger, has the same appearance.

Cell Propagation. Mitosis. While mitotic figures are easily recognisable, it must be remembered that the whole process is a continuous one, and that the early changes in the nucleus, between the interphase and the prophase, are very easily overlooked. These changes may completely alter the apparent structure of the nucleus

to 40 microns in diameter. The nucleus usually appears as a group of separate lobes each of which has a definite nuclear membrane and is filled with fine dust like particles. The cytoplasm has no definite rim and is filled with small moderately bright granules.

(I) *Platelets*. These appear most as small circles of light 2 to 5 microns in diameter. The larger number appear to be empty but some contain five or six small bright granules which show active Brownian movement apparently bouncing from side to side of the cell.

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(Q) *Late Normoblast*. Seven to 8 microns in diameter with a thick refractile rim.

(R) *Normocyte*
thick cytoplasmic rim
same appearance

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(4) **ERYTHROBLASTIC SERIES** (M) *Promegaloblast*. A non-motile cell about 10 microns in diameter. The cytoplasmic rim is thick and well defined. This cell shows marked pleomorphism. The cytoplasm appears completely free of granules and other structures although there may be a group of small rounded mitochondria adherent to one side of the nucleus, when this occurs, the nuclear membrane is absent at the point of contact. This membrane is otherwise well defined and thin. The nucleus is filled with fine dust like particles which are more closely packed and appear much brighter than those of the leucoblastic cells. Also the nuclear particles show some small areas of condensation which appear as brighter granules. The nucleoli, two or three in number, are outlined by a ring of such condensation.

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(P) *Early Normoblast*. The nuclear structure is the same as in the megaloblast, and the nucleus is of much the same size. The cytoplasmic ring is not well marked, and the cytoplasm is scanty and may contain a few dust like mitochondria. The cell is easily recognised by its nuclear structure.

(Q) *Late Normoblast*. Seven to 8 microns in diameter, with a thick refractile rim.

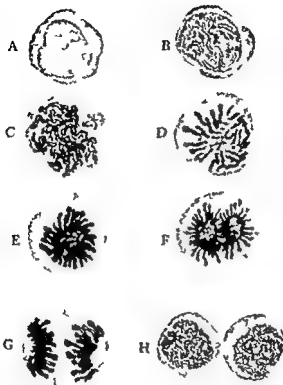
(R) *Normocyte*.
thick cytoplasmic rim
same appearance

Cell Propagation. Mitosis. While mitotic figures are easily recognisable, it must be remembered that the whole process is a continuous one, and that the early changes in the nucleus, between the interphase and the prophase, are very easily overlooked. These changes may completely alter the apparent structure of the nucleus

and cause some confusion as to the actual nature of the cell. The same remarks apply to the final transition from the telophase to the interphase, except that the presence of two daughter cells, lying in apposition, should give a clue to the real condition. Failure to appreciate these changes, especially in histological material where there is much cell shrinkage, has led to much of the confusion which has existed over the morphological characters of blood cells and their apparent relationships.

The advent of sternal puncture has made it possible to study these changes much more closely, and they are illustrated on Plate 14. The cells depicted here are promonocytes from a case of monocytic leukaemia. Care has been taken to choose typical figures such as will be seen in normal haemopoiesis. One of the characteristics of a leukaemia is, of course, the presence of atypical mitoses, tri- and even tetrapolar figures can be seen.

In the resting cell (A, Plate 14) the nucleus is rounded and the chromatin shows a fine, rather irregular reticulation. The cytoplasm has a ground glass appearance. In the earliest stage of the prophase the pattern of the nucleus becomes more distinct owing to the disappearance of small anastomosing links, leaving the permanent chromatic elements of the chromosomes, the chromonema (B, Plate 14). It is at this stage that the greatest care is required in labelling a cell, a lymphoblast in this stage of development might easily be mistaken for a myeloblast. Some authors have described such changes in monocytes, and used them to suggest that the cell was derived from a lymphocyte. In the later stage of the prophase (C, Plate 14) the chromosomes are beginning to appear as separate entities. Each consists of two chromonemata lying parallel and fixed in an achromatic matrix. During the metaphase this matrix stains intensely and the internal structure of the chromosome is no longer visible. The arrangement of the chromosomes in the metaphase appears to be constant for all the cells of the haemopoietic tissue. The long chromosomes are arranged radially at the periphery, and the short ones lie irregularly in the centre (E, Plate 14). In the anaphase, the chromonema from each chromosome move to opposite poles so that each daughter nucleus will have a representative part of the parent chromosome. The anaphase is shown in Fig F, Plate 14. In the telophase the matrix of each daughter nucleus becomes achromatic again and the chromosome structure is again visible (G, Plate 14). Further changes in the new nuclei are the same as in the prophase, only occurring in the reverse order (H, Plate 14).



Mitotic Division ($\times 2000$)
(See p. 85)

The cytoplasm divides by furrowing. It undergoes quite definite changes during the nuclear division, changing from a fine homogeneous structure to a mass of fine interwoven threads (Plate 14). The cytoplasm of dividing erythroblasts and normoblasts appears to be granular rather than thread-like.

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